HETEROCYCLES, Vol. 98, No. 2, 2019, pp. 175 - 203. © 2019 The Japan Institute of Heterocyclic Chemistry
Received, 2nd December, 2018, Accepted, 4th February, 2019, Published online, 22nd February, 2019
DOI: 10.3987/REV-18-901

# ADVANCES IN THE SYNTHESIS OF 1,2,4-TRIAZOLO[1,5-*a*][1,3,5]TRIAZINES (5-AZAPURINES) AND THEIR BIOLOGICAL ACTIVITY

**Koon Kee Kow[a] and Anton V. Dolzhenko[a,b]***

[a] School of Pharmacy, Monash University Malaysia, Jalan Lagoon Selatan, Bandar Sunway, Selangor Darul Ehsan 47500, Malaysia, anton.dolzhenko@monash.edu

[b] School of Pharmacy and Biomedical Sciences, Curtin Health Innovation Research Institute, Faculty of Health Sciences, Curtin University, GPO Box U1987 Perth, Western Australia 6845, Australia, anton.dolzhenko@curtin.edu.au

**Abstract** – The 1,2,4-triazolo[1,5-*a*][1,3,5]triazine system has been recognized as a promising scaffold for drug development purposes. The isosteric relationship between this skeleton and purines plays an important role in the research towards the development of bioactive 1,2,4-triazolo[1,5-*a*][1,3,5]triazine derivatives. This review discusses recently developed strategies for the synthesis of 1,2,4-triazolo[1,5-*a*][1,3,5]triazines and advances achieved in studies on biological activity of compounds constructed using this skeleton.

## CONTENTS

## 1. INTRODUCTION

Purine isosters have been attracting attention of researchers in the medicinal chemistry field as promising scaffolds for the construction of bioactive compounds.[1] Their structural similarity to the most ubiquitous in nature *N*-heterocycle, purine, opens an opportunity to target purinome consisting of purine-binding proteins. Approximately 13% of the human genome is involved in coding these proteins, many of which are druggable.[2] Therefore, potential therapeutic applications of molecules targeting purinome are very diverse.

Various azolotriazines have been explored as purine isosters in many drug discovery programs.[3,4] Among azolo-1,3,5-triazines, the 1,2,4-triazolo[1,5-*a*][1,3,5]triazine system has been one of the most extensively studied scaffolds recognized as promising for the discovery of new drugs.[4] The 1,2,4-triazolo[1,5-*a*][1,3,5]triazine structure possesses all nitrogen atoms of purine in their original positions but has one more nitrogen atom replacing the carbon atom in position 5 of purine ring system and therefore is referred to as 5-azapurine (Figure 1).

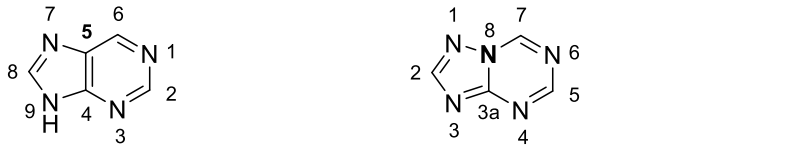



Figure 1

Since the latest review[5] on 1,2,4-triazolo[1,5-*a*][1,3,5]triazines, the information published on the synthesis and biological activity of these compounds has nearly doubled. New interesting methods for the synthesis of these compounds have been developed and a substantial progress has been made in investigations of their biological activities. Herein we systematize new findings in this continuously developing area covering advances in the field since the previous comprehensive review published in 2006.[5]

## 2. SYNTHESIS OF 1,2,4-TRIAZOLO[1,5-*a*][1,3,5]TRIAZINES

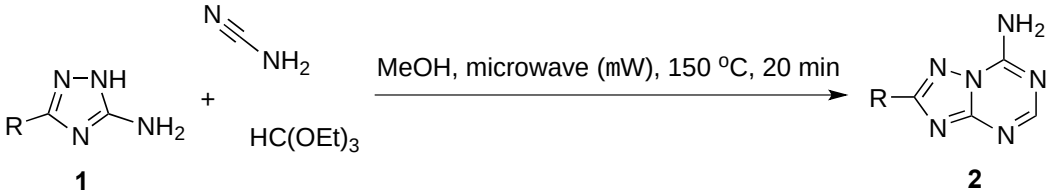
Two principal approaches are used to construct the 1,2,4-triazolo[1,5-*a*][1,3,5]triazine system: the fusion of the 1,3,5-triazine ring to the 1,2,4-triazole moiety and the annelation of the 1,2,4-triazole ring to the 1,3,5-triazine one. In this review, we discuss synthetic approaches to 1,2,4-triazolo[1,5-*a*][1,3,5]triazines in this order followed by a separate section dedicated to polyfused systems incorporating 1,2,4-triazolo[1,5-*a*][1,3,5]triazine skeleton in their structure.

### 2.1. 1,3,5-Triazine ring formation in the synthesis of 1,2,4-triazolo[1,5-*a*][1,3,5]triazines

The most convenient building blocks used this strategy were 5(3)-amino-1,2,4-triazoles or intermediates derived from them. In the 1,3,5-triazine ring annulation, 5(3)-amino-1,2,4-triazoles played a role of

binucleophilic reagent. Usually, these reactions of 5(3)-amino-1,2,4-triazoles were rather regioselective and resulted in the triazine ring closure at the N1 atom of triazoles affording 1,2,4-triazolo[1,5-*a*][1,3,5]triazines.

Inspired by the close resemblance of the reaction product to biogenic adenine, a one-pot, three-component synthesis of 5-azaadenine (**2a**) was developed using the reaction of 5-amino-1,2,4-triazole (**1a**) with cyanamide and triethyl orthoformate (Scheme 1).[6] The reaction gave satisfactory outcome only under high temperature generated by the microwave heating. When this reaction was performed under reflux in the same solvent, it produced a complex mixture of products with only traces of **2a**. It was found that sequential introductions of the reagents to the reaction were not beneficial regardless order of their addition. The scope of the reaction was further explored and this method was applied for the synthesis of libraries of substituted 5-azaadenines (Scheme 1).[6,7]

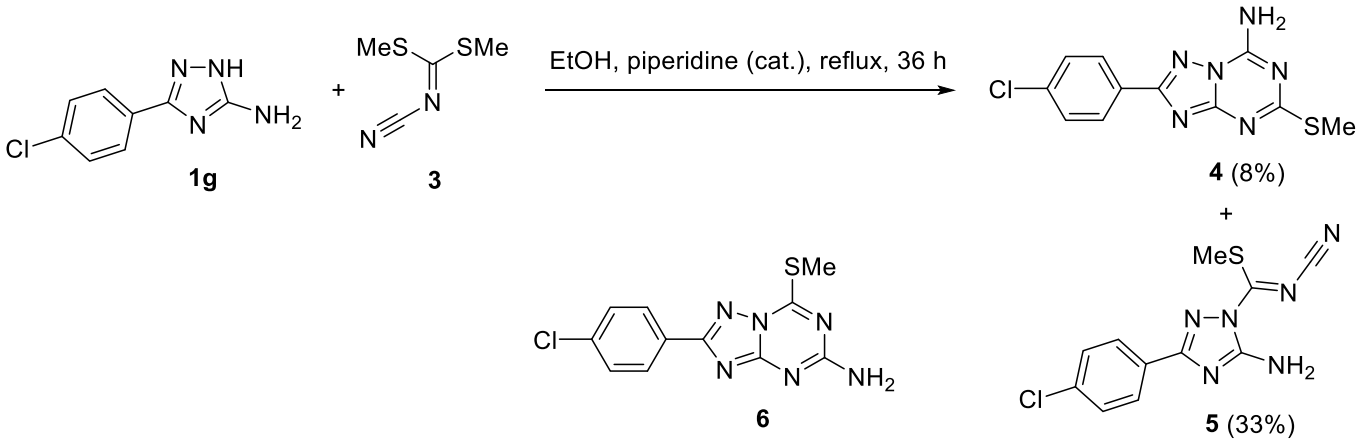



R = H, 65% (**2a**); Ph, 68% (**2b**); 3-$FC_6H_4$, 72% (**2c**); 4-$FC_6H_4$, 88% (**2d**); 2-$ClC_6H_4$, 65% (**2e**); 3-$ClC_6H_4$, 56% (**2f**); 4-$ClC_6H_4$, 73% (**2g**); 3-$NO_2C_6H_4$, 59% (**2h**); 4-$MeC_6H_4$, 85% (**2i**); 3-$MeOC_6H_4$, 64% (**2j**); 4-$CF_3C_6H_4$, 56% (**2k**); 2-furyl, 71% (**2l**); 2-thienyl, 76% (**2m**); 3-Py, 70% (**2n**); 4-Py, 58% (**2o**); PhNH, 75% (**2p**); 4-$FC_6H_4NH$, 73% (**2q**); 4-$ClC_6H_4NH$, 74% (**2r**); 3-$MeC_6H_4NH$, 70% (**2s**); 4-$MeC_6H_4NH$, 72% (**2t**); 3-$MeOC_6H_4NH$, 73% (**2u**); 4-$MeOC_6H_4NH$, 70% (**2v**); 3-$CF_3C_6H_4NH$, 75% (**2w**); NHBn, 52% (**2x**); $NHCH_2CH_2Ph$, 55% (**2y**); morpholino, 75% (**2z**); SMe, 74% (**2aa**).

Scheme 1

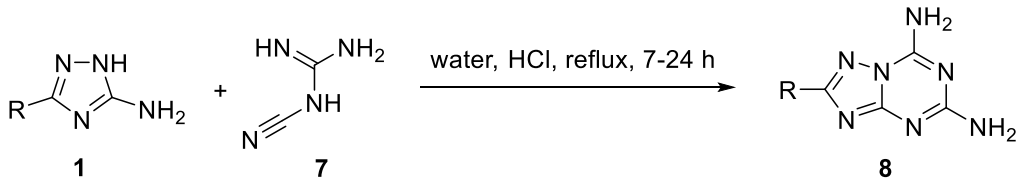
Several C-N-C synthons were used in reactions with 5(3)-amino-1,2,4-triazoles (**1**) for the formation of the fused 1,3,5-triazine ring.

The earlier explored by Caulkett et al.[8] reaction of 5(3)-amino-1,2,4-triazoles (**1**) with *S,S*-dimethyl *N*-cyanodithiocarbonimidate (**3**) was performed under modified conditions (reflux in ethanol in the presence of catalytic quantities of piperidine).[9] Two products were isolated from the reaction mixture and the 1,2,4-triazolo[1,5-*a*][1,3,5]triazine structure **4** was suggested for one of them, while the second one was proposed to be triazole **5** (Scheme 2). It should be noted that **4** was isolated in the rather low yield and no sufficient experimental data were provided to support structure assignments. The lack of comprehensive structural analysis and data of earlier reports[10] indicate that formation of regioisomeric products, *i.e.* **6** (instead of **4**) cannot be excluded.




Scheme 2

The synthesis of 5,7-diamino-1,2,4-triazolo[1,5-*a*][1,3,5]triazines **8** was effectively performed by the heating of 5(3)-amino-1,2,4-triazoles **1** with cyanoguanidine (**7**) in aqueous media (Scheme 3).[9,11,12] The reaction was facilitated by hydrochloric acid, but can be also carried out without acid by heating the reagents in DMF (*e.g.* **8j**, R = 4-Py, 50% - 4 h).[11] The nitration of **8e** by nitric acid was reported[12] to occur at the amino groups in positions 2 and 5 affording an explosive derivative, which structure was established by X-ray crystallography.

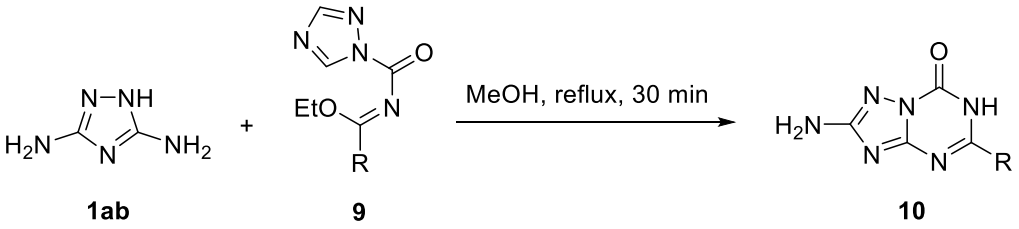



R = H, 70% (**8a**); Ph, 64% (**8b**); 4-ClC$_6$H$_4$, 75% (**8c**); Bn, 52% (**8d**); NH$_2$, (**8e**); NHPh, 55% (**8f**); NHBn, 68% (**8g**); NHCH$_2$CH$_2$Ph, 65% (**8h**); SMe, 57% (**8i**).

Scheme 3

The reaction of guanazole (**1ab**) with *N*-(1,2,4-triazol-1-ylcarbonyl)imidates **9** afforded 2-amino-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-7-ones **10** (Scheme 4).[13] The 1,3,5-triazine ring closure proceeded selectively and structures of the products were confirmed spectroscopically and by the X-ray crystallography study of **10f**.[13]

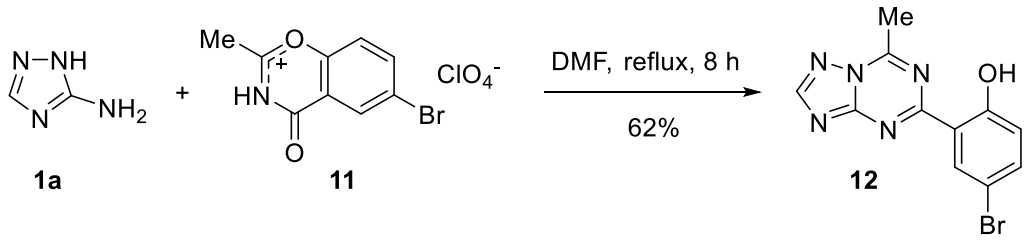
It was reported that heating 5-amino-1,2,4-triazole (**1a**) with 6-bromo-2-methyl-4-oxobenz[1,3-*e*]oxazinium perchlorate (**11**) in DMF resulted in the opening of the oxazine ring followed by the selective recyclization of the triazine ring thus affording 1,2,4-triazolo[1,5-*a*][1,3,5]triazine **12** (Scheme 5).[14]




**1ab** **9** **10**

R = *cyclo*-Hex, 77% (**10a**); 4-$ClC_6H_4$, 60% (**10b**); 4-$ClC_6H_4CH_2$, 75% (**10c**); 3,4-$Cl_2C_6H_3CH_2$, 78% (**10d**); 3,4-$(MeO)_2C_6H_3CH_2$, 52% (**10e**); $PhCH_2CH_2$, 67% (**10f**).

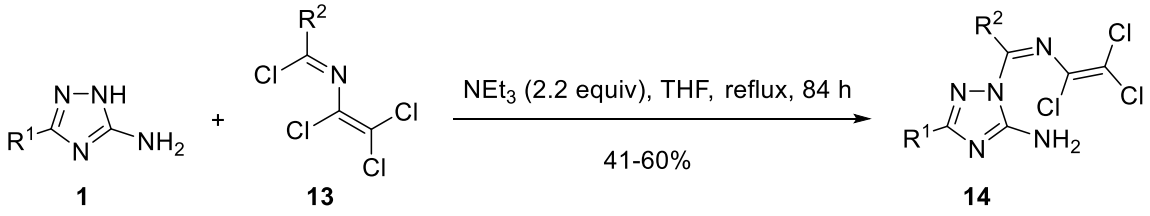
Scheme 4




**1a** **11** **12**

Scheme 5

It was demonstrated that *N*-trichlorovinyl-substituted imidoyl chlorides **13** reacted with the N1 endocyclic atom of 5(3)-amino-1,2,4-triazoles (**1**) in the presence of base (Scheme 6).[15] The isolated intermediates **14** underwent intramolecular ring closure upon their treatment with sodium methoxide. The yields of 5-dichloromethyl-substituted **15** over the two steps were in the range of 35-53%. The structures of products **15** were confirmed spectroscopically and by X-ray crystallographic data for **15b**.[15]

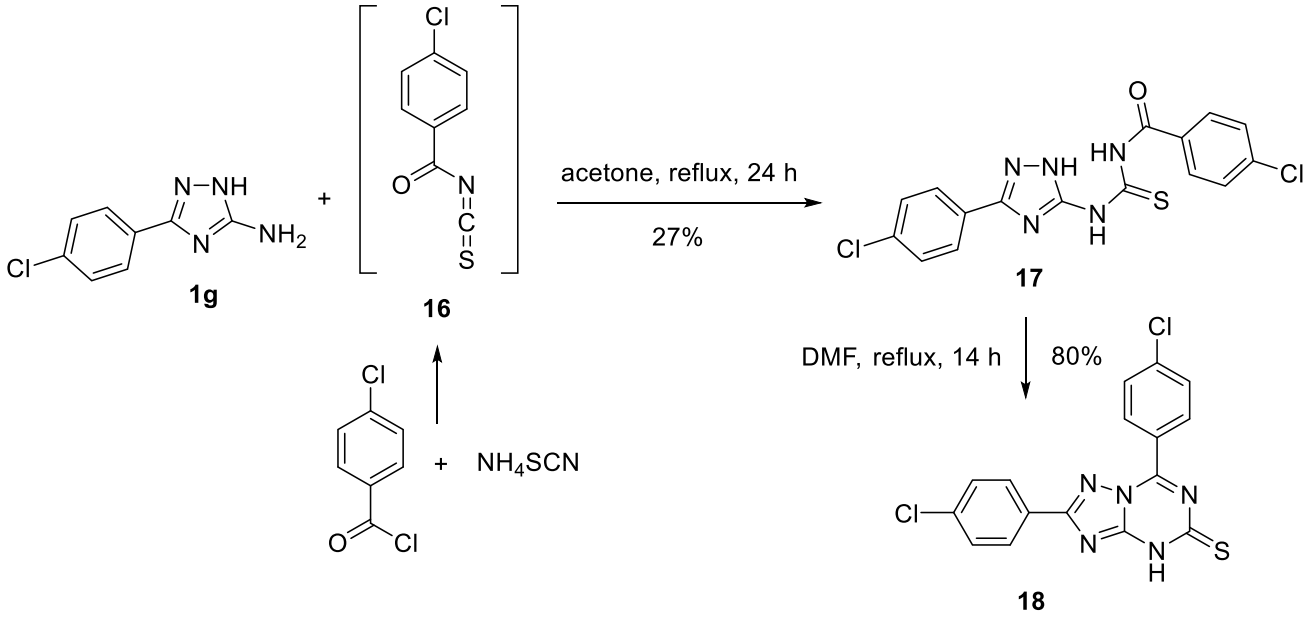



**1** **13** **14** **15**

| **15** | $R^1$ | $R^2$ | Yields, % |
|---|---|---|---|
| **a** | SMe | Ph | 43 |
| **b** | SMe | 4-$MeC_6H_4$ | 53 |
| **c** | $CF_3$ | Ph | 40 |
| **d** | $CF_3$ | 4-$MeC_6H_4$ | 35 |
| **e** | Ph | Ph | 45 |
| **f** | Ph | 4-$MeC_6H_4$ | 48 |

Scheme 6

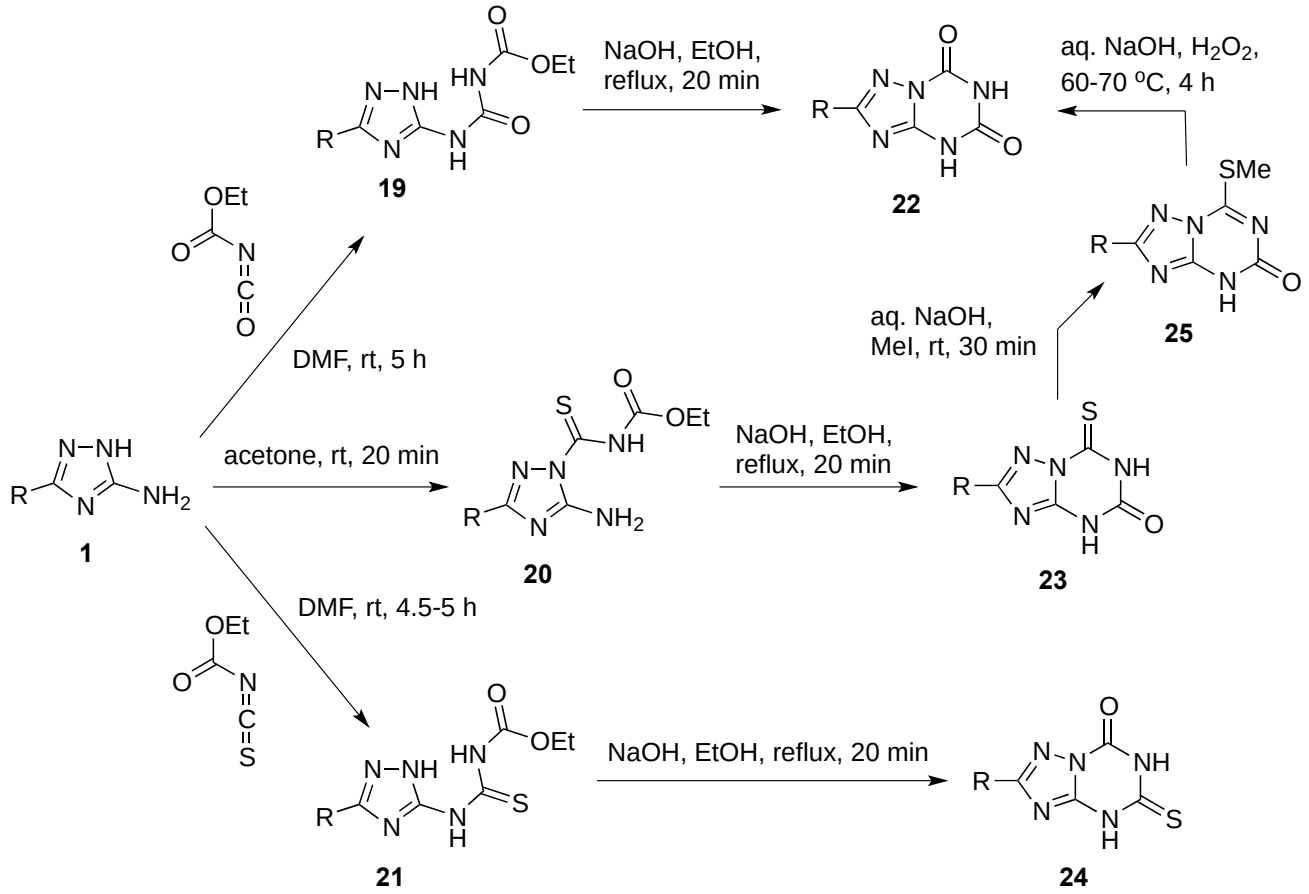
The reaction of aminotriazole **1g** with 4-chlorobenzoyl isothiocyanate (**16**) (preformed from 4-chlorobenzoyl chloride and ammonium thiocyanate) was reported to result in the formation of 1-(4-chlorobenzoyl)-3-(3-(4-chlorophenyl)-1,2,4-triazol-5-yl)thiourea (**17**), which upon heating in DMF underwent intramolecular cyclization to afford 1,2,4-triazolo[1,5-*a*][1,3,5]triazine-5-thione **18** (Scheme 7).[9]




Scheme 7

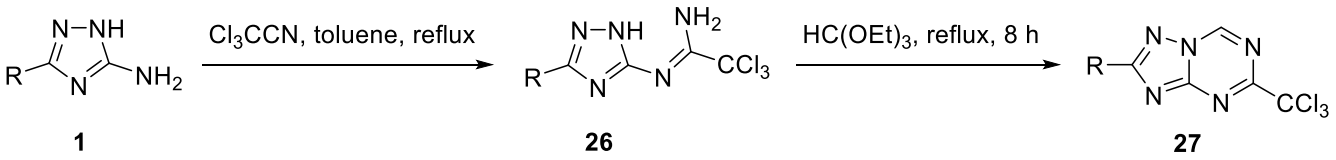
In the search for new inhibitors of thymidine phosphorylase, a series of 5-azaxanthine derivatives **22** and their thiocarbonyl analogues **23** and **24** were prepared (Scheme 8).[16-19] The synthesis of 5-azaxanthines **22** was achieve by the base-catalyzed intramolecular cyclization of urea **19**[16] and alternatively *via* the oxidation-hydrolysis of the methylsulfanyl group of **25**.[17] Two types of regioisomeric thiocarbonyl-substituted 1,2,4-triazolo[1,5-*a*][1,3,5]triazinones **23** and **24** were prepared by the triazine ring closure of the corresponding intermediates **20** and **21**, which were synthesized using the reactions of 5(3)-amino-1,2,4-triazoles **1** with ethoxycarbonyl isotiocyanate under the kinetic or thermodynamic control, respectively. The substantial biological activity (*vide infra* Section 3.2) was found only for 5-thioxo-5,6-dihydro-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-7(4*H*)-ones **24** and an extensive library of these compounds bearing diverse substituents on the triazole ring was prepared using this method.[16-19]

The synthesis of 5-trichloromethyl-substituted 1,2,4-triazolo[1,5-*a*][1,3,5]triazines **27** was achieved in the two-step process (Scheme 9).[20] The initial addition of aminotriazoles **1** to the triple-bond of trichloroacetonitrile resulted in the formation of trichloroacetamidines **26**, which underwent cyclization upon heating in triethyl orthoformate.




**22** (from **19**): R = Ph, 54% (**22a**); Bn, 47%(**22b**) [ref. 16]
**22** (from **25**): R = Ph, 54% (**22a**); SMe, 68%(**22c**) [ref. 17]
**23**: R = Ph, 59% (**23a**); SMe, 72%(**23b**) [ref. 17]
**24**: 43 examples - 40-94% [refs. 16-19]

Scheme 8

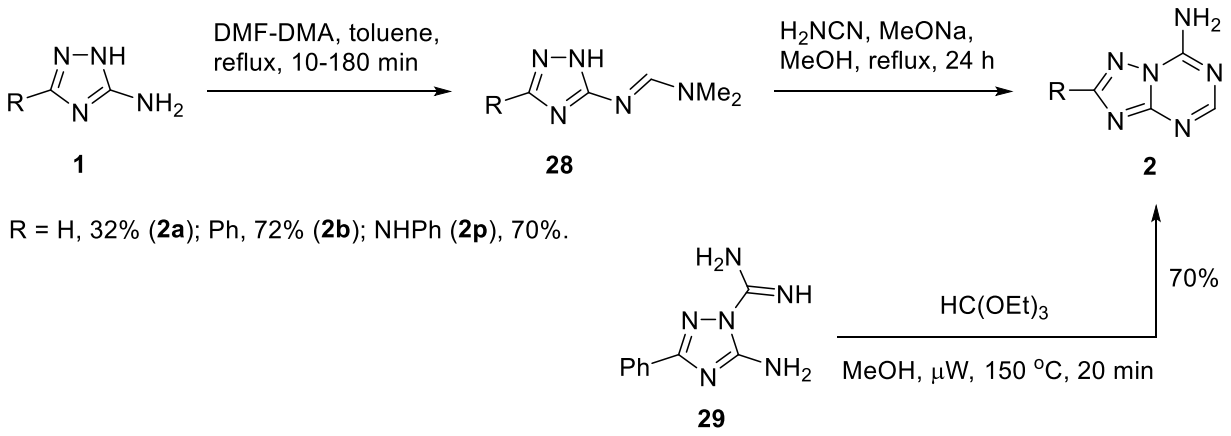



R = Ph, 72% (**27a**); 3-$MeOC_6H_4$, 76% (**27b**); 4-$ClC_6H_4$, 56% (**27c**).

Scheme 9

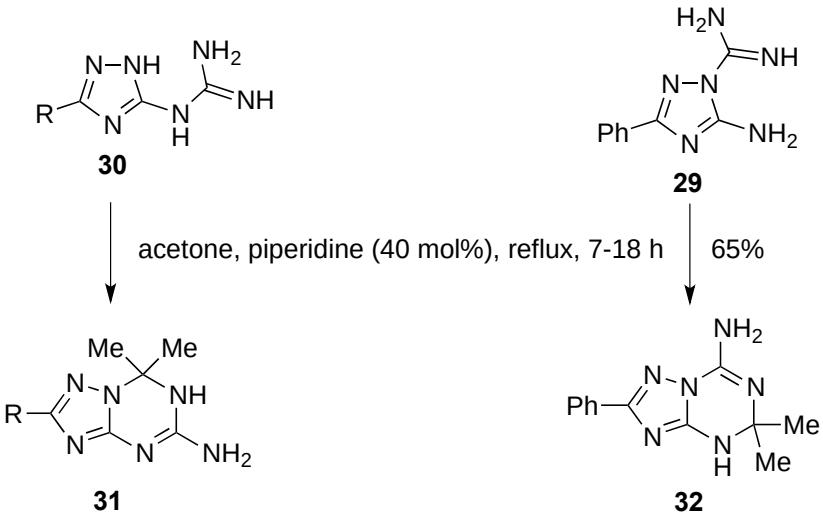
An efficient two-step synthesis of 5-azaadenines **2** from 5(3)-amino-1,2,4-triazoles **1** was developed (Scheme 10).[21] It the first step, 5(3)-amino-1,2,4-triazoles **1** were converted to the corresponding formamidines **28** (71-96% yields) by the treatment with *N*,*N*-dimethylformamide dimethyl acetal (DMF-DMA). Then, intermediates **28** reacted with cyanamide in the presence of sodium methoxide to afford 5-azaadenines **2**. In another method for the synthesis of 8-phenyl-5-azaadenine (**2b**), triethyl

orthoformate was used as a one-carbon inserting reagent in the reaction with 5-amino-1-guanyl-3-phenyl-1,2,4-triazole (**29**) under microwave heating in methanol.[6]



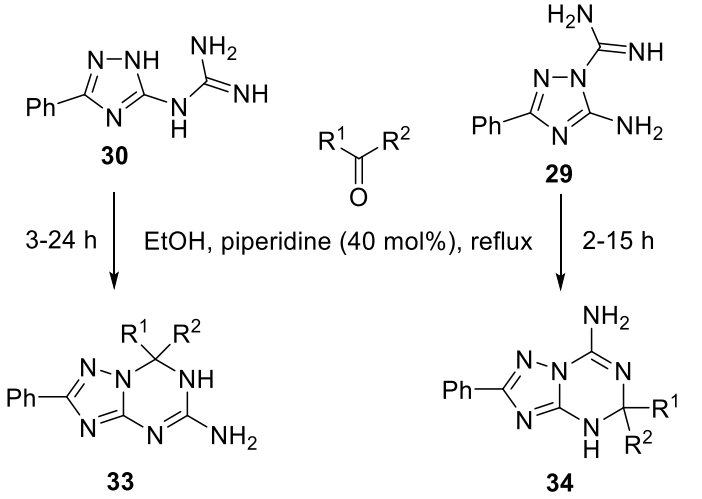

Scheme 10

It was found that heating 5-guanidino-1,2,4-triazoles **30** in acetone in the presence of base *i.e.* piperidine resulted in the formation of 5-amino-7,7-dimethyl-6,7-dihydro-1,2,4-triazolo[1,5-*a*][1,3,5]triazines **31** (Scheme 11).[22,23] The triazine ring closure proceeded selectively to the N1 atom of the triazole and the 6,7-dihydro-form was found to be the principal tautomeric form of the products in solution (confirmed by NMR spectroscopy) and solid (X-ray crystallography) states.[24,25] The product **32** with regioisometic substitution pattern on the triazine ring was prepared similarly starting from 5-amino-1-guanyl-3-phenyl-1,2,4-triazole (**29**).[22] The detail analysis of the structure of **32** was carried out using X-ray crystallography.[26]




Scheme 11

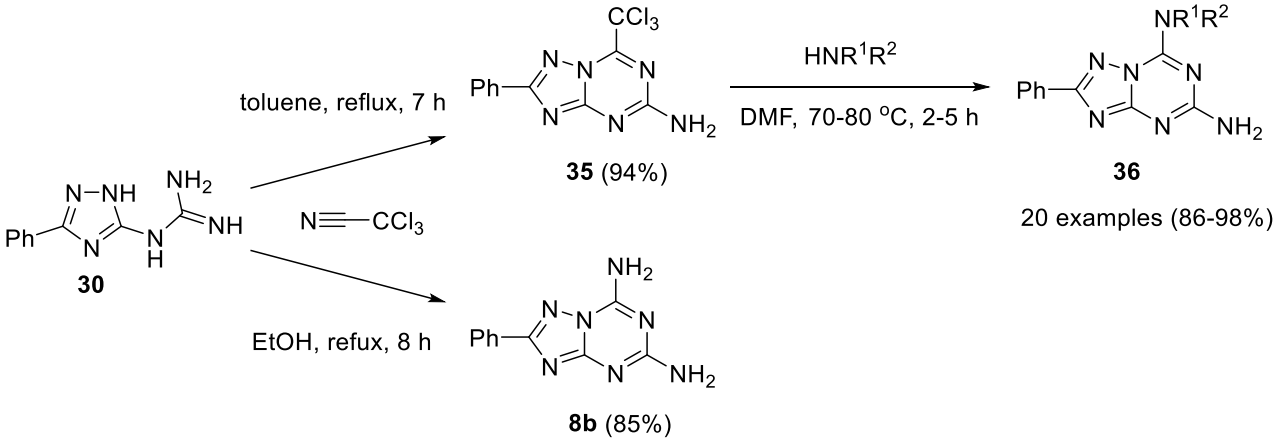
These methodologies were extended to the synthesis of two series of regioisomeric 5-amino-6,7-dihydro- and 7-amino-4,5-dihydro-1,2,4-triazolo[1,5-*a*][1,3,5]triazines **33** and **34** using base-catalyzed reactions of **30** and **29** with aldehydes or ketones (Scheme 12).[22]




| **33** | Yields, % | $R^1$ | $R^2$ | **34** | Yields, % |
|---|---|---|---|---|---|
| **b** | 92 | H | Ph | **b** | 78 |
| **c** | 91 | H | 4-$MeOC_6H_4$ | **c** | 87 |
| **d** | 90 | H | 4-$MeC_6H_4$ | **d** | 72 |
| **e** | 73 | H | 4-$ClC_6H_4$ | **e** | 70 |
| **f** | 76 | H | 4-$FC_6H_4$ | **f** | 78 |
| **g** | 72 | H | 2-Furyl | **g** | 70 |
| **h** | 78 | H | 4-Py | **h** | 68 |
| **i** | 96 | $(CH_2)_4$ | | **i** | 62 |
| **j** | 72 | $(CH_2)_5$ | | **j** | 73 |

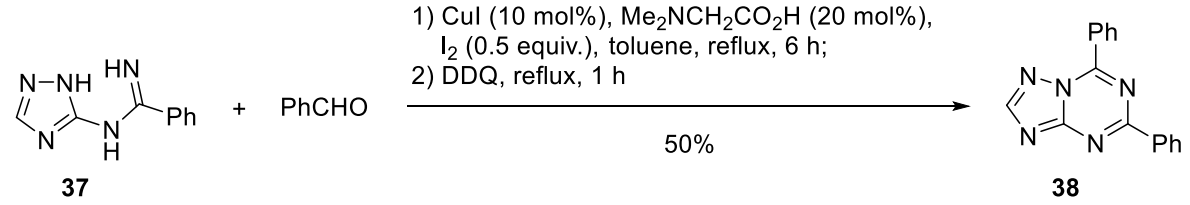
Scheme 12

Further research on the synthesis of 1,2,4-triazolo[1,5-*a*][1,3,5]triazines from triazolylguanidines **30** established that the reactions of **30** with trichloroacetonitrile was solvent-dependent.[27] It was found that the reaction performed in toluene resulted in the exclusive formation of 5-amino-7-trichloromethyl-2-phenyl-1,2,4-triazolo[1,5-*a*][1,3,5]triazine (**35**), which was found to be an excellent substrate for ammination by the nucleophilic substitution of the trichloromethyl group (Scheme 13). The structure of the resulting **36** was confirmed spectroscopically and studied in details by X-ray crystallography of a representative compound **36a** ($R^1 = R^2 = Me$).[28] However, the same reaction of **30** with trichloroacetonitrile in ethanol produced 5,7-diamino-2-phenyl-1,2,4-triazolo[1,5-*a*][1,3,5]triazine (**8b**) only.



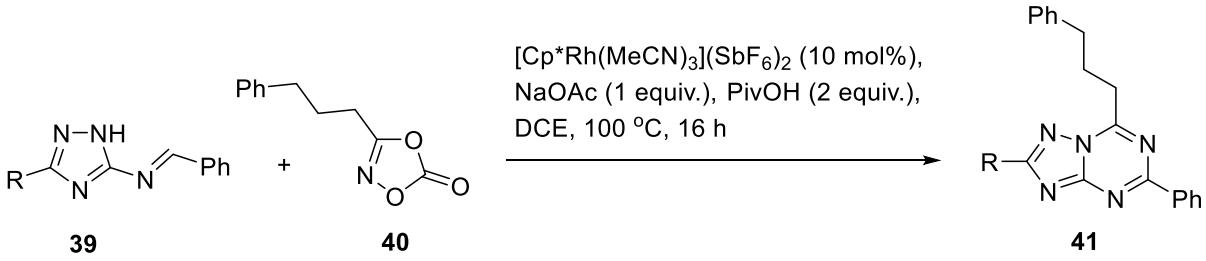

Scheme 13

The Cu(I)/$I_2$-catalyzed triazine ring closure of amidine **37** with benzaldehyde was reported to involve a metallated intermediate and *in situ* oxidation by DDQ thus resulting in the formation of 5,7-diphenyl-1,2,4-triazolo[1,5-*a*][1,3,5]triazine (**38**) (Scheme 14).[29]




Scheme 14

An interesting reaction of 5-(*N*-benzylideneamino)-1,2,4-triazoles **39** with 1,4,2-dioxazol-5-one **40** was reported by Ellman's research group (Scheme 15).[30] Proceeding *via* an initial Rh(III)-catalyzed C-H amidation of **39** by **40**, this reaction furnished the triazine ring closure by the cyclodehydration affording 1,2,4-triazolo[1,5-*a*][1,3,5]triazines **41**.

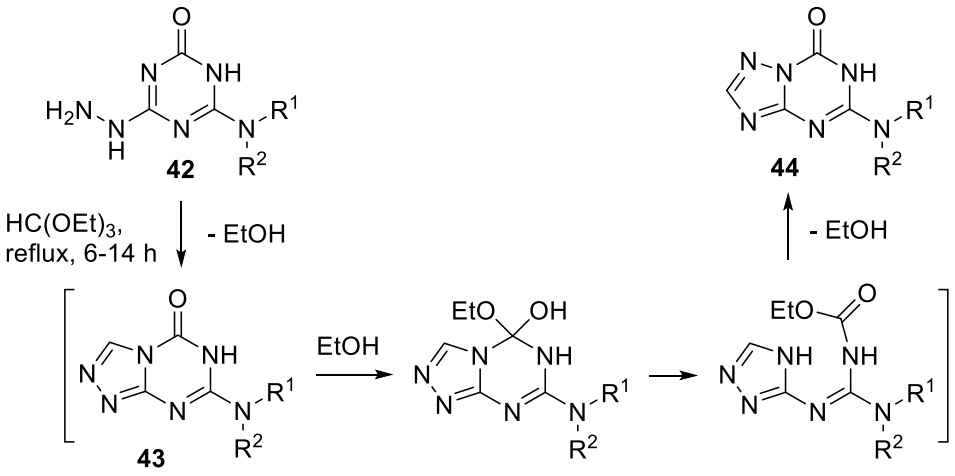



R = Me, 57% (**41a**); Ph, 56% (**41b**).

Scheme 15

## 2.2. 1,2,4-Triazole ring formation in the synthesis of 1,2,4-triazolo[1,5-*a*][1,3,5]triazines

The syntheses of 1,2,4-triazolo[1,5-*a*][1,3,5]triazines by the triazole ring annulation typically utilize as starting materials hydrazino-substituted 1,3,5-triazines and involve the Dimroth rearrangement of the isomeric 1,2,4-triazolo[4,3-*a*][1,3,5]triazines formed as intermediates. It was reported that the hydazino group of 1,3,5-triazin-2-ones **42** reacted with triethyl orthoformate and upon the triazine ring closure afforded 5-azaguanines **44** in good yields (Scheme 16).[31] The initially formed 1,2,4-triazolo[4,3-*a*][1,3,5]triazines **43** were suggested to undergo the transformation to their [1,5-*a*]-fused analogues **44** *via* an instant Dimroth rearrangement facilitated by ethanol released at the cyclocondensation step. The structures of the products formed were also confirmed by X-ray crystallography data for compounds **44a** and **44g**.[31] A selective alkylation at position 3 of 5-amino-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-7-ones **44** was also performed.[32]

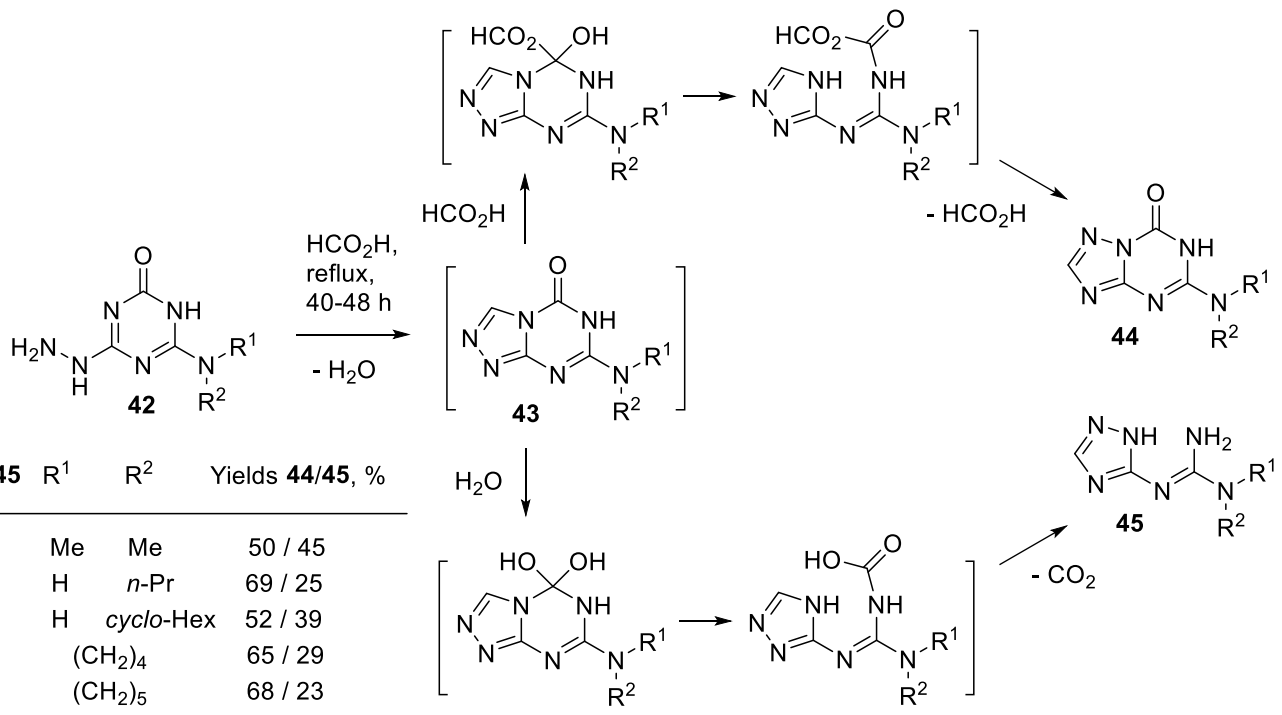



| 44 | $R^1$ | $R^2$ | Yields, % |
|---|---|---|---|
| **a** | Me | Me | 75 |
| **b** | H | *n*-Pr | 85 |
| **c** | H | *i*-Pr | 80 |
| **d** | H | *cyclo*-Hex | 90 |
| **e** | H | Ad | 84 |
| **f** | | $(CH_2)_4$ | 82 |
| **g** | | $(CH_2)_5$ | 75 |

Scheme 16

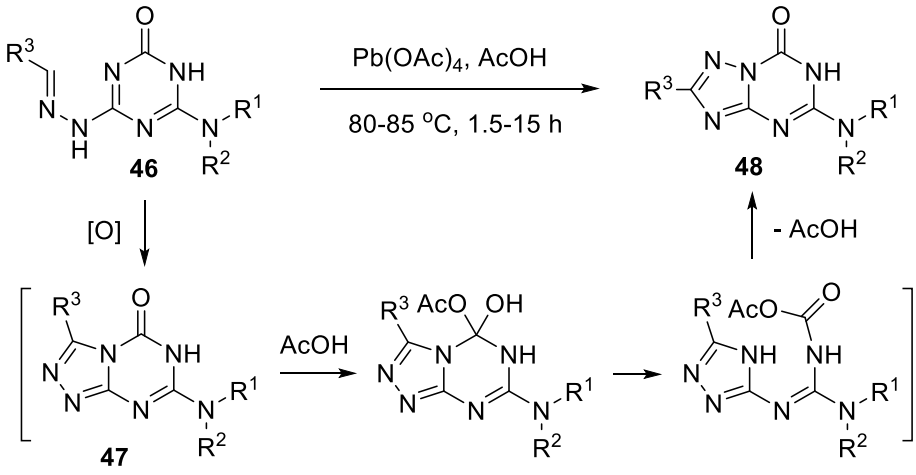
When 1,3,5-triazin-2-ones **42** were heated in formic acid, the reaction outcome was found to depend on the presence of water in the reaction mixture.[33] Together with expected 5-amino-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-7-ones **44**, *N*-triazolylguanidines **45** were isolated from the reaction mixture (Scheme 17). These observations were explained by the proposed mechanism, which suggested that water competed with formate in the nucleophilic attack of the carbonyl group in intermediates **43** during the Dimroth rearrangement step. Experimentally, the proposed mechanism was supported by reactions using aqueous formic acid as a medium: an increase of water content dramatically shifted the ratio of products towards guanidines **45** until they became sole products. Additional confirmation of the suggested pathway came from an attempt to perform hydrolysis of 1,2,4-triazolo[1,5-*a*][1,3,5]triazin-7-ones **44a** in 80% aqueous formic acid (reflux for 48 h). No signs of guanidine **45a** were detected in the reaction mixture and only starting material **44a** was isolated, thus indicating that water played its role in directing reaction outcome at the rearrangement step. The structure of **45a** was studied using X-ray crystallography.[33]

The synthesis of 2-substituted analogues of **44**, compounds **48**, was performed using an oxidative ring closure of hydrazones **46**, which were prepared from **42**.[34] The treatment of **46** with lead(IV) tetracetate resulted in the selective triazole ring closure to the nitrogen atom adjacent to the carbonyl group, while acetic acid, used as a solvent, facilitated the Dimroth rearrangement of intermediate **47** to the targeted products **48** (Scheme 18). The structures of **48** were confirmed spectroscopically and by X-ray crystallography for **48d**.[34]




| **44**, **45** | $R^1$ | $R^2$ | Yields **44**/**45**, % |
|---|---|---|---|
| **a** | Me | Me | 50 / 45 |
| **b** | H | *n*-Pr | 69 / 25 |
| **d** | H | *cyclo*-Hex | 52 / 39 |
| **f** | $(CH_2)_4$ | | 65 / 29 |
| **g** | $(CH_2)_5$ | | 68 / 23 |

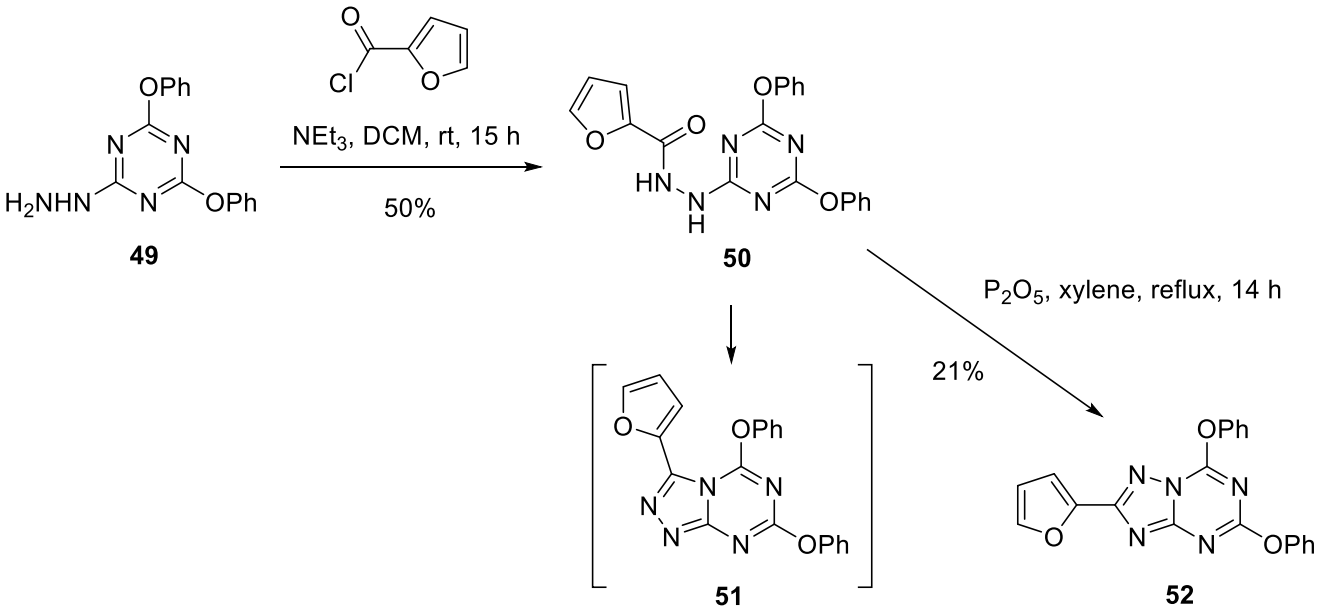
Scheme 17




| **48** | $R^1$ | $R^2$ | $R^3$ | Yields, % |
|---|---|---|---|---|
| **a** | Me | Me | Ph | 61 |
| **b** | H | *n*-Pr | Ph | 49 |
| **c** | H | *i*-Pr | Ph | 53 |
| **d** | $(CH_2)_4$ | | Ph | 60 |
| **e** | $(CH_2)_5$ | | Ph | 56 |
| **f** | $CH_2CH_2OCH_2CH_2$ | | Ph | 52 |
| **g** | $CH_2CH_2OCH_2CH_2$ | | 4-$BrC_6H_4$ | 50 |
| **h** | Me | Me | 5-$NO_2$-2-furyl | 74 |
| **i** | $(CH_2)_4$ | | 5-$NO_2$-2-furyl | 67 |
| **j** | $(CH_2)_5$ | | 5-$NO_2$-2-furyl | 54 |

Scheme 18

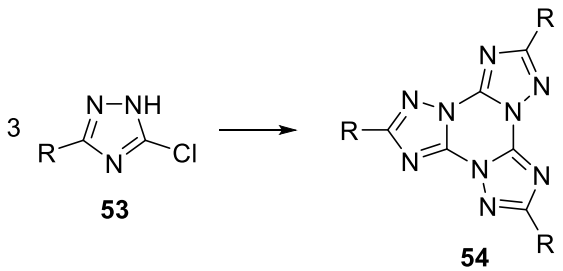
The synthesis of 2-(furan-2-yl)-5,7-diphenoxy-1,2,4-triazolo[1,5-*a*][1,3,5]triazine (**52**) was achieved *via* a phosphorus pentoxide promoted thermal dehydrative 1,3,5-triazine ring closure of **50** prepared from 2-hydrazino-4,6-diphenoxy-1,3,5-triazine (**49**) (Scheme 19).[35] The formation of **52** involved the Dimroth rearrangement of the [4,3-*a*]-fused isomer **51**.[8] It was reported that **52** could be used as a useful building block for the synthesis of adenosine receptor antagonists.[35]




Scheme 19

### 2.3. Synthesis of 1,2,4-triazolo[1,5-*a*][1,3,5]triazines fused with other heterocyclic rings

Tartakosvky et al.[36] reported the synthesis of tris[1,2,4]triazolo[1,5-*a*:1',5'-*c*:1'',5''-*e*][1,3,5]triazines **54** by thermal cyclization of 5(3)-chloro-1,2,4-triazoles **53** (Scheme 20).

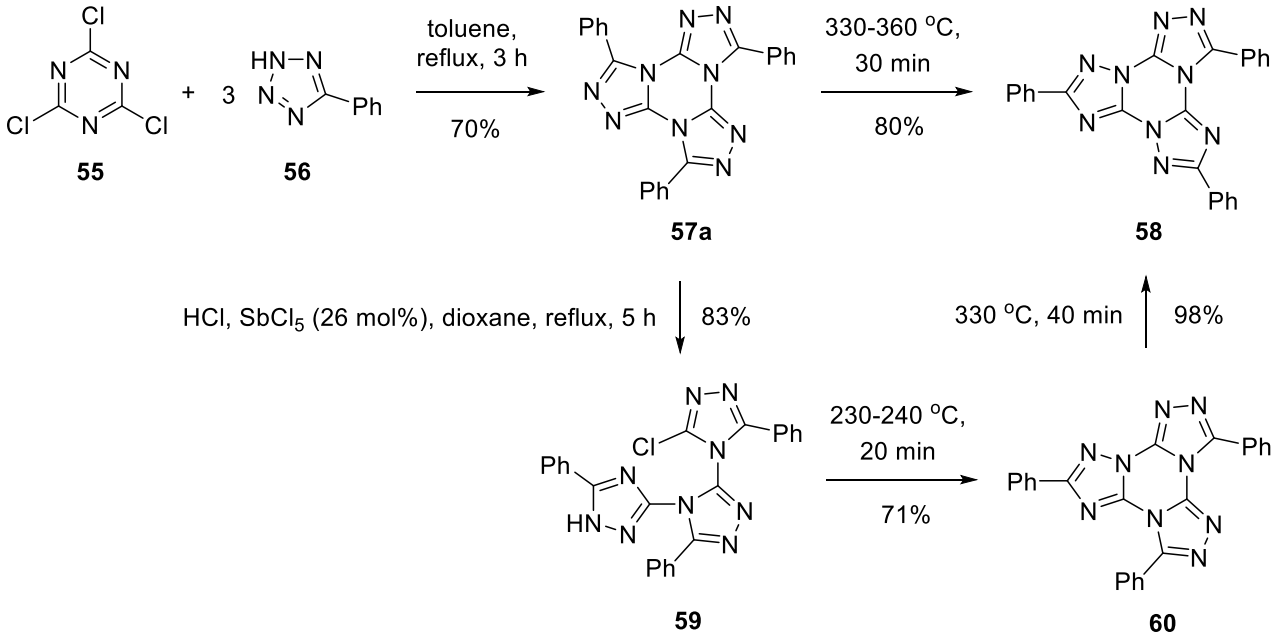



| **54** | R | Conditions | Yield, % |
|---|---|---|---|
| **a** | Cl | sulfolane, 250-270 °C, 2 h | 20 |
| **b** | Ph | 230-270 °C, 1 h | 46 |

Scheme 20

The regioisomeric 3,7,11-triphenyltris[1,2,4]triazolo[4,3-*a*:4',3'-*c*:4'',3''-*e*][1,3,5]triazine (**57a**) was prepared by the Huisgen's method[37] from cyanuric chloride (**55**) and 5-phenyltetrazole (**56**) (Scheme 21). The thermolysis of **57a** at 330-360 °C led to the Dimroth rearrangement at two triazole rings to afford

3,6,10-triphenyltris[1,2,4]triazolo[1,5-*a*:1',5'-*c*:4",3"-*e*][1,3,5]triazine (**58**) in the good yield. The analogue **60** with one triazole ring in the [1,5-*a*] junction was obtained by the intramolecular thermal cyclization of **59**, which was prepared *via* the triazine ring opening upon the treatment of **57a** with hydrochloric acid in the presence of antimony pentachloride. At higher temperature, **60** almost quantitatively rearranged to **58**.



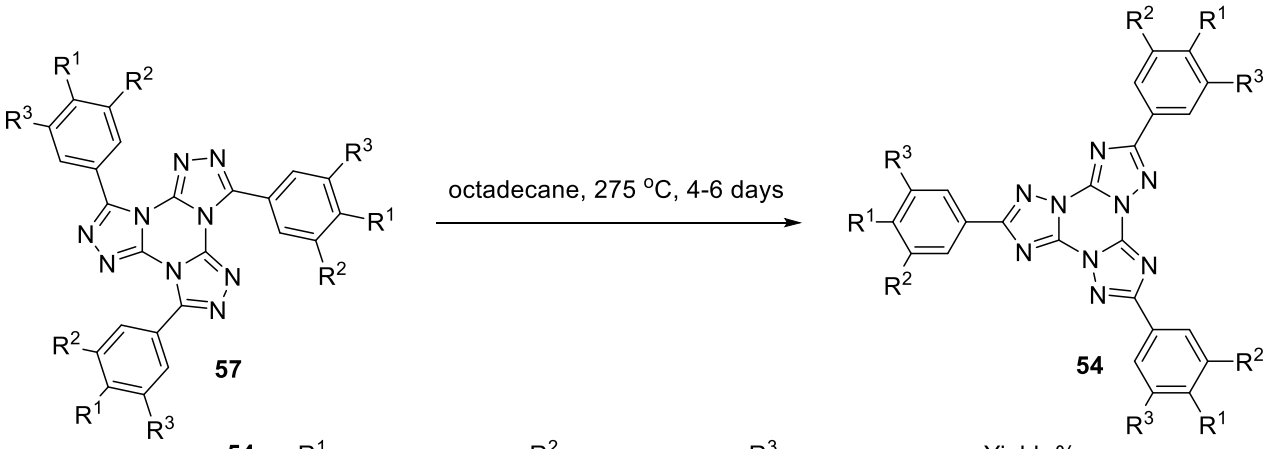

**55** **56** **57a** **58** **59** **60**

Scheme 21

The rearrangement at all three triazole rings even at lower temperature was reported for a series of 3,7,11-triaryltris[1,2,4]triazolo[4,3-*a*:4',3'-*c*:4",3"-*e*][1,3,5]triazines **57** with some alkoxy substituents on the phenyl rings, but the reaction required several days of heating in an inert solvent under exclusion of oxygen (Scheme 22).[38] The time-dependent NMR spectroscopy revealed that the rearrangement of all three triazole rings proceeded in a step-wise manner with relatively fast rearrangement of the first triazole ring. The structure of the products was confirmed spectoscopically, including $^{15}$N NMR spectroscopy, and by X-ray crystallography of **54c**.[38] It was also found that **54** with peripheral alkoxy chains were able to form discotic liquid crystals with interesting properties.

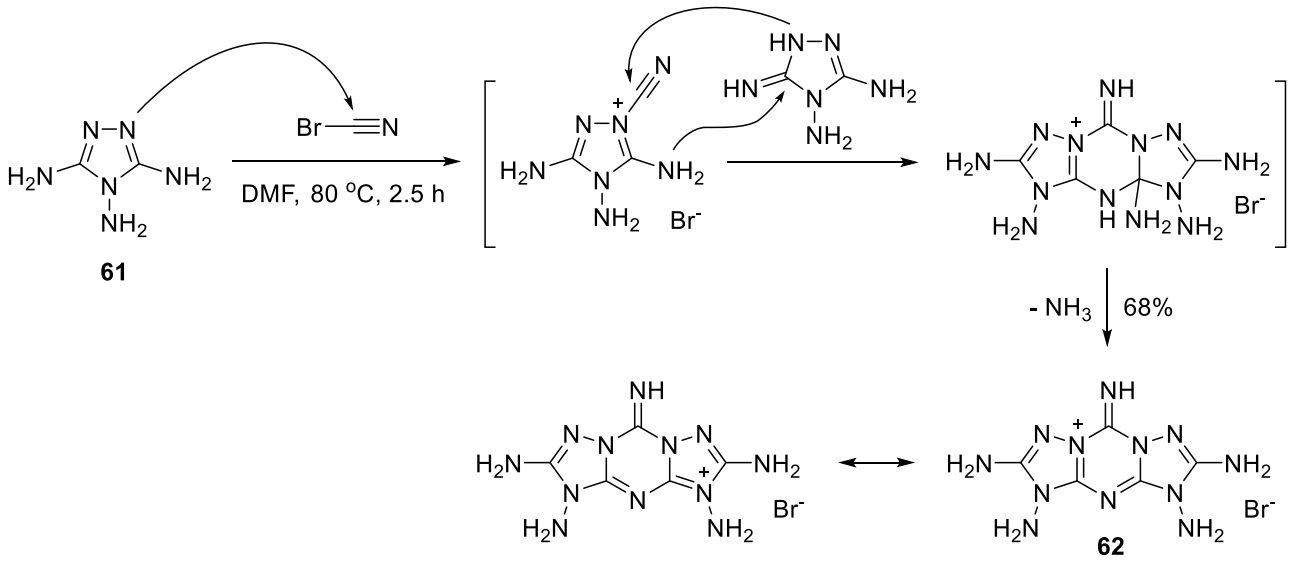
An interesting synthesis of tricyclic system **62**, consisting of two 1,2,4-triazole rings fused to the 1,3,5-triazine ring, was performed using the reaction of guanazine (**61**) with cyanogen bromide (Scheme 23).[39] It was proposed that the reaction started with the selective cyanation at the N1 endocyclic nitrogen of one guanazine molecule. Then, the process involved another guanazine molecule, presumably as an imine tautomer, initiating the 1,3,5-triazine ring closure followed by the elimination of ammonia to afford

**62**. The analysis of spectroscopic and X-ray crystallographic data indicated that positive charge in the 3*H*,9*H*-bis[1,2,4]triazolo[1,5-*a*:5'1'-*d*][1,3,5]triazinium system was not symmetrically delocalized between both triazole rings.[39]




| **54** | $R^1$ | $R^2$ | $R^3$ | Yield, % |
|---|---|---|---|---|
| **c** | $OC_3H_7$ | H | H | 65 |
| **d** | $OC_{10}H_{21}$ | $OC_{10}H_{21}$ | H | 43 |
| **e** | $OC_{12}H_{25}$ | $OC_{12}H_{25}$ | H | 38 |
| **f** | $OC_{14}H_{29}$ | $OC_{14}H_{29}$ | H | 18 |
| **g** | $OC_{16}H_{33}$ | $OC_{16}H_{33}$ | H | 21 |
| **h** | $OC_8H_{17}$ | $OC_8H_{17}$ | $OC_8H_{17}$ | 26 |
| **i** | $OC_{10}H_{21}$ | $OC_{10}H_{21}$ | $OC_{10}H_{21}$ | 22 |
| **j** | $O(CH_2)_3CH(Et)Bu$ | $O(CH_2)_3CH(Et)Bu$ | $O(CH_2)_3CH(Et)Bu$ | 32 |

Scheme 22




Scheme 23

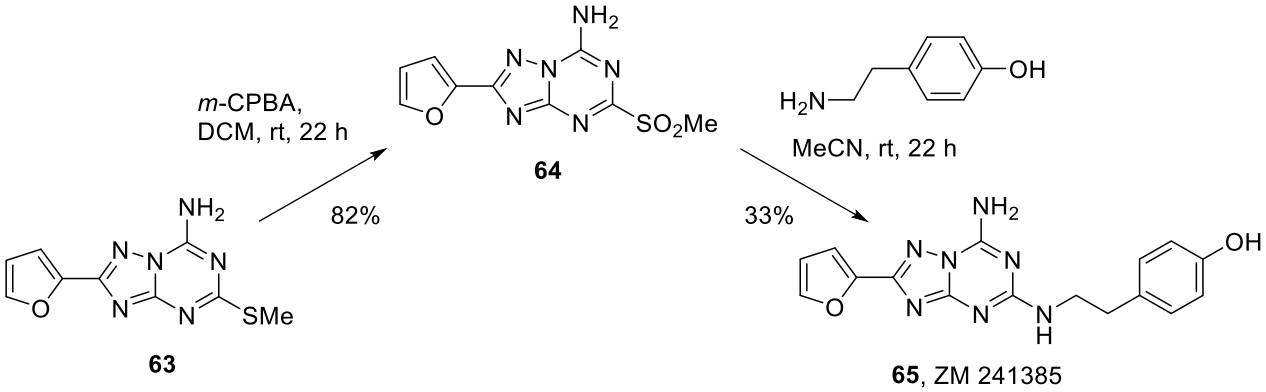
## 3. BIOLOGICAL ACTIVITY OF 1,2,4-TRIAZOLO[1,5-*a*][1,3,5]TRIAZINE DERIVATIVES

The spectrum of biological activities of 1,2,4-triazolo[1,5-*a*][1,3,5]triazines is very wide,[1,4,5] but recent advancements were made mainly in the development of new adenosine receptor antagonists. The achievements in this area and other data on biological activities of 1,2,4-triazolo[1,5-*a*][1,3,5]triazines are discussed below.

### 3.1. Adenosine receptor antagonists

The 1,2,4-triazolo[1,5-*a*][1,3,5]triazine scaffold has been extensively applied for the construction of ligands for adenosine receptors, particularly $A_{2a}$ adenosine receptors, which have been identified as a valid therapeutic target, especially for the Parkinson disease treatment.[40,41]

For the last decade, a selective $A_{2a}$ adenosine receptor antagonist ZM 241385 (**65**) has become an invaluable tool for the research on adenosine receptors, purinergic signaling and related fields. It is the most commonly used in pharmacological experiments standard $A_{2a}$ adenosine receptor antagonist. For the detailed structural studies,[42] ZM 241385 (**65**) was synthesized according to the earlier described general methodology[43] by the nucleophilic displacement of the methylsulfonyl group of **64**, which was prepared *via* the oxidation of **63** by *m*-chloroperoxybenzoic acid (*m*-CPBA), with tyramine (Scheme 24). The spectroscopic properties of **63**, **64** and **65**, particularly their concentration and temperature dependent NMR spectra, were discussed together with the X-ray crystallographic data for **65**.[42] The X-ray crystallographic studies on ZM 241385 (**65**) crystallized in complex with $A_{2a}$ adenosine receptor were also performed using various technics.[44-54]

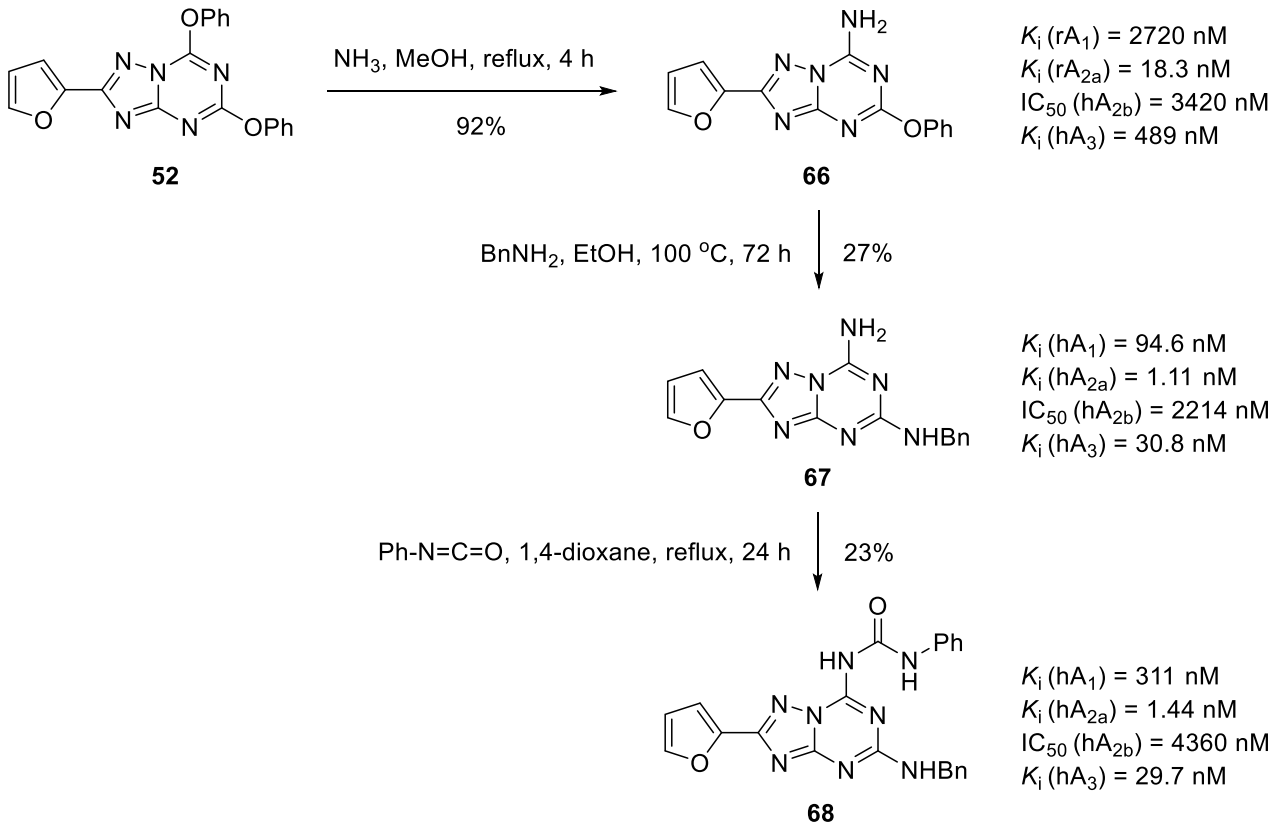



Scheme 24

In the search of new, potent and selective $A_{2a}$ adenosine receptor antagonists, a series of 5,7-diamino-2-(furan-2-yl)-1,2,4-triazolo[1,5-*a*][1,3,5]triazines, *e.g.* compound **67**, were prepared by consecutive amminations in positions 5 and 7 *via* nucleophilic substitutions of the phenoxy groups at **52** (Scheme 25).[35] The resulting products possessing a primary amino group at position 7 were further

derivatized at this group to generate substituted amides or ureas (*e.g.* **68**). The benzylamino substituted **67** was found to be a rather potent and selective ligand for $A_{2a}$ adenosine receptors. Even higher selectivity was achieved upon the treatment of **67** with phenyl isocyanate leading to the conversion of the primary amino group into the corresponding phenylurea **68**, which was the most promising in this series. It should be noted that the 5-phenoxy substituted intermediate **66** and its amide and urea derivatives also demonstrated relatively good affinity and selectivity towards $A_{2a}$ adenosine receptors.[55] The similar compounds with methylsulfanyl or dimethylamino group at position 5 of the 1,2,4-triazolo[1,5-*a*][1,3,5]triazine skeleton were significantly less active.

The modifications of the general scaffold of ZM 241385 (**65**) are commonly performed at the amino group in position 5 of the 1,2,4-triazolo[1,5-*a*][1,3,5]triazine system. These structural changes aimed to tune affinity and selectivity of ligands, increase their aqueous solubility,[56] as well as modify kinetics of their binding to the receptors and type of the binding.[57]



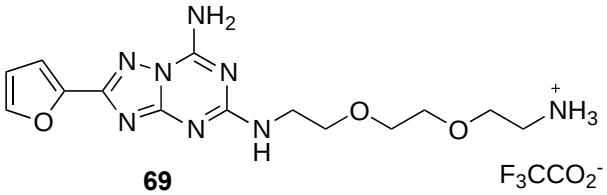

Scheme 25

The nucleophilic substitution of the phenoxy group at the position 5 of **66** was used for the preparation of compounds possessing better aqueous solubility.[56] Two most promising compounds identified in this work[56] were trifluoroacetates **69** and **70**, which demonstrated an excellent affinity and selectivity towards

$A_{2a}$ adenosine receptors and were readily soluble in water at concentrations up to 10 mM.

Another library of 24 analogues of ZM 241385 (**65**) with basic amino groups in the side-chain replacing the tyramine moiety was prepared using an approach similar to the one illustrated in Scheme 24.[57] These compounds were very selective towards $A_{2a}$ adenosine receptors and their kinetics of binding to these receptors was explored. It was found that *N*-phenylpiperazine moiety connected to the triazine ring *via* an ethyleneamino linkage was particularly beneficial for the residence time (RT) of the ligand at the binding site. The structure modifications of the prototype molecule **71** on the phenyl ring identified that RT can be further increased by 2,4-difluoro substituents on the phenyl. The RT for the resulting compound **75** (323 min) was 4.5 times longer than the RT for ZM 241385 (**65**) (71 min). The X-ray crystallographic data obtained for the complexes of $A_{2a}$ adenosine receptor with ZM 241385 (**65**) and its four analogues **72-75** suggested that the difference in RTs could be attributed to their influence on the stability of the Glu169-His264 salt bridge in the receptor.[50] Both ZM 241385 (**65**) and **75** stabilized this interaction in the receptor, while **72**, **73**, and **74** weakened it.

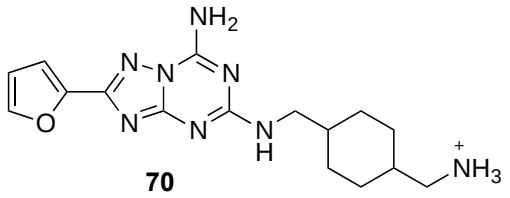
The similar synthetic approach was utilized for the preparation of the covalent antagonists for $A_{2a}$ adenosine receptors.[58,59] On the basis of molecular modeling and experimental data from the site-directed mutagenesis experiments, the reactive fluorosulfonyl group of the designed ligand LUF7445 (**76**) was proposed to bind covalently to lysine amino acid K153 of the receptor.[58] In continuation of this work, compounds with an extended polymethylene linkage between the 1,2,4-triazolo[1,5-*a*][1,3,5]triazine core and the electrophilic centre were prepared.[59] The most active derivative was further modified to introduce a propargyl handle, thus making the obtained product LUF7487 (**77**) the first clickable affinity-based probe for $A_{2a}$ adenosine receptors. It was demonstrated that $A_{2a}$ adenosine receptors covalently linked to LUF7487 (**77**) could be efficiently visualized by the copper-catalyzed azide-alkyne ligation with a fluorophore. The results of experiments, utilizing LUF7487 (**77**) for labeling $A_{2a}$ adenosine receptors in cell membrane, suggested that this probe could be a useful tool for pharmacological studies of $A_{2a}$ adenosine receptors.



**69**

$F_3CCO_2^-$

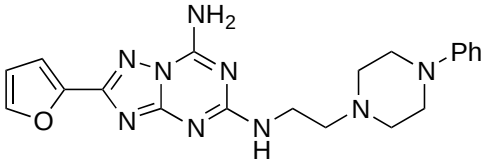
$K_i$ ($hA_1$) = 769 nM
$K_i$ ($hA_{2a}$) = 11.5 nM
$IC_{50}$ ($hA_{2b}$) > 10,000 nM
displ. at 10 mM ($hA_3$) = 59.2%



**70**

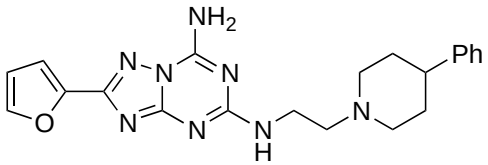
$F_3CCO_2^-$

$K_i$ ($hA_1$) = 1350 nM
$K_i$ ($hA_{2a}$) = 16.9 nM
$IC_{50}$ ($hA_{2b}$) = 10,700 nM
displ. at 10 mM ($hA_3$) = 72.8%



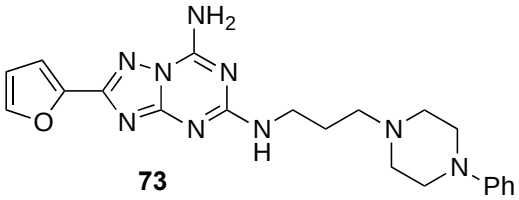
**71**

$K_i$ ($hA_{2a}$) = 0.3 nM, RT = 164 min
$K_i$ ($hA_1$) > 1000 nM



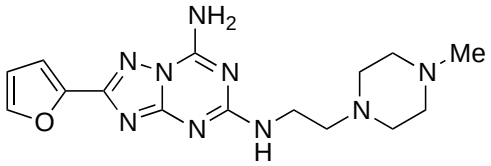
**72**

$K_i$ ($hA_{2a}$) = 3.8 nM, RT = 3 min
$K_i$ ($hA_1$) > 1000 nM



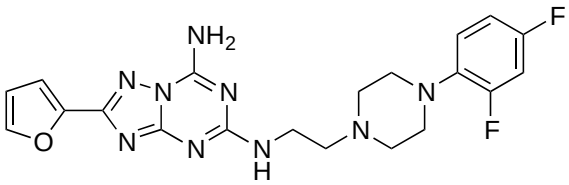
**73**

$K_i$ ($hA_{2a}$) = 1.3 nM, RT = 4 min
$K_i$ ($hA_1$) > 1000 nM



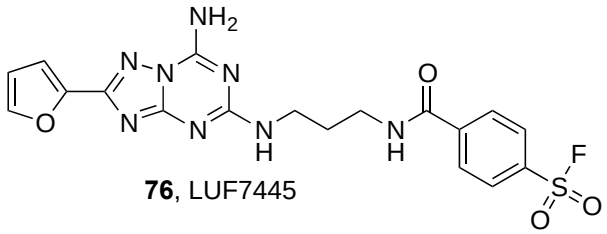
**74**

$K_i$ ($hA_{2a}$) = 31 nM, RT = 4 min
$K_i$ ($hA_1$) > 1000 nM

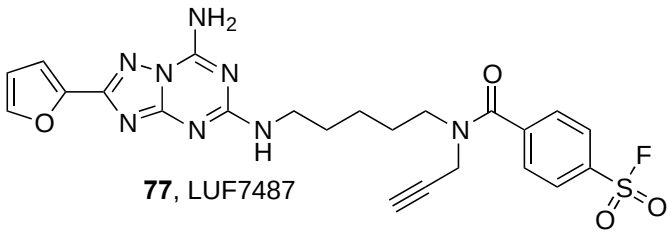


**75**

$K_i$ ($hA_{2a}$) = 0.33 nM, RT = 323 min
$K_i$ ($hA_1$) > 1000 nM



**76**, LUF7445



**77**, LUF7487

Figure 2

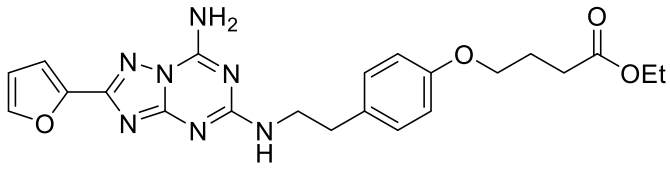
A number of experiments were carried out to identify the position and type of linkers, which can be attached to ZM 241385 (**65**) without substantial loss of activity and could be potentially used to link another bioactive molecule. It was demonstrated that substitution at the phenol group of ZM 241385 (**65**)

were well tolerated due to its orientation in the binding site towards extracellular environment. The promising results were obtained with compound **78** possessing a butyrate moiety at the phenolic oxygen (Figure 3).[60] In the $A_{2a}$ adenosine receptor functional assay, **78** demonstrated activity comparable to that of ZM 241385 (**65**) ($IC_{50}$ = 42 nM). This strategy was used to link a molecule of ZM 241385 with a $D_2$ dopamine receptor agonist ropinirole in compound **79**, possessing excellent activity towards both $A_{2a}$ adenosine receptors (*cf*. ZM 241385's $IC_{50}$ ($hA_{2a}$) = 33 nM) and $D_2$ dopamine receptors (*cf*. ropinirole's $EC_{50}$ ($hD_2$) = 304 nM).[61] The incorporation of an $A_{2a}$ adenosine receptor antagonist and a $D_2$ dopamine receptor agonist into one molecule was performed to increase therapeutic potential of the compounds for the treatment of Parkinson's disease. Another interesting heterobivalent ligand combining ZM 241385 and ropinirole *via* a morpholino-1,3,5-triazine linker was **80**, which possessed a lower activity towards the targeted receptors but demonstrated an excellent *in vitro* brain : blood partition ratio of 3.64. When the ZM 241385 pharmacophore was nor completely incorporated into the structure of compounds, the resulting integrated dual acting ligands were substantially less active against $A_{2a}$ adenosine receptors but their agonistic properties towards $D_2$ dopamine receptor were improved in some cases, *e.g*. for **81**. The brain : blood partition ratio for **81** was greater than that of ZM 241385 (**65**): 1.29 vs. 0.76, respectively. The therapeutically beneficial properties of **81** also included low protein binding.

In the attempt to create a useful for the Parkinson's disease treatment prodrug of an $A_{2a}$ adenosine receptor antagonist **70** and dopamine, these molecules were linked *via* a succinamide bridge in compound **82** (Figure 3).[62] It was found that **82** retained good affinity and selectivity to $A_{2a}$ adenosine receptors, including those in rat striatum. A rather beneficial hydrolysis profile was observed for **82**. In whole blood, the hydrolysis occurred preferentially at the linker amide group connected to the $A_{2a}$ adenosine receptor antagonist side and thus preventing peripheral side effects of dopamine. Contrary, in rat brain homogenates, the hydrolysis took place exclusively at the dopamine side of the succinamide linker and allowed controlled release of dopamine in the CNS.

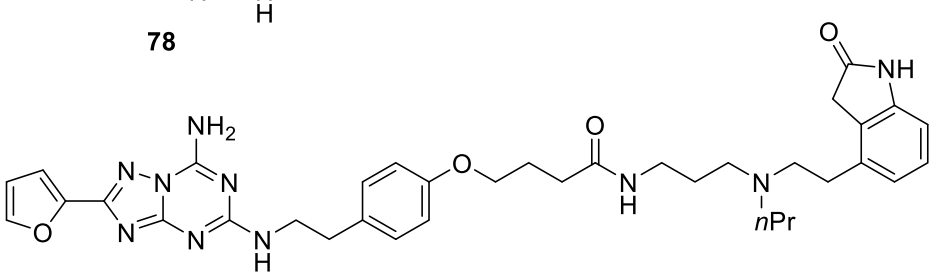
### 3.2. Enzyme inhibitors

In the search for new antibiotics, 1,2,4-triazolo[1,5-*a*][1,3,5]triazine **85** was prepared by the treatment of aminotriazole **83** with ethyl *N*-cyanoacetimidate (**84**) in the mixture of dimethoxyethane (DME) and *N*-methylpyrrolidone (NMP) (Scheme 26).[64] It was reported the **85** inhibited phosphopantetheine adenylyltransferase (PPAT) from Gram-negative bacteria. This compound (**85**) inhibited PPAT from *Escherichia coli* and *Pseudomonas aeruginosa* with $IC_{50}$ values 5.9 and 4.6 μM, respectively. However, the activity was significantly improved by removing one nitrogen atom from the triazine ring (changing the scaffold to 1,2,4-triazolo[1,5-*a*]pyrimidine system).



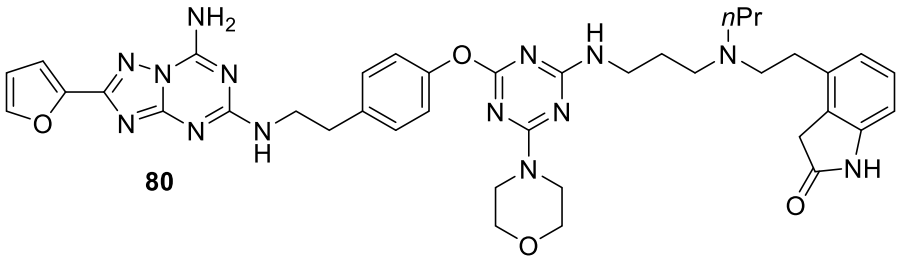
**78**

$IC_{50}$ ($hA_{2a}$) = 50 nM



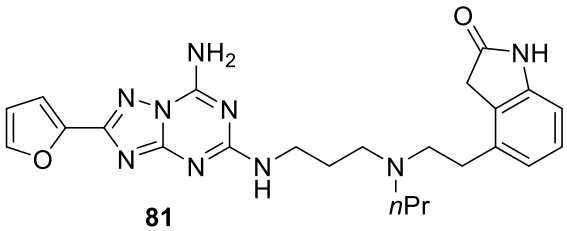
**79**

$IC_{50}$ ($hA_{2a}$) = 18 nM
$EC_{50}$ ($hD_2$) = 204 nM



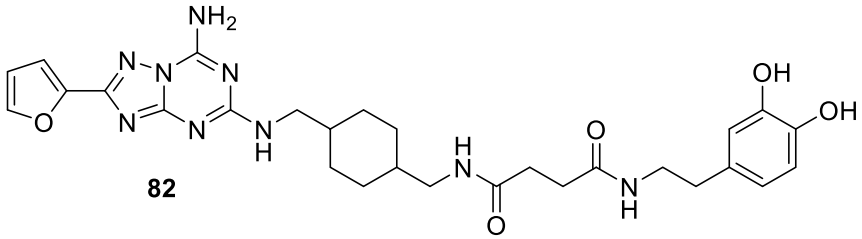
**80**

$IC_{50}$ ($hA_{2a}$) = 120 nM
$EC_{50}$ ($hD_2$) = 311 nM



**81**

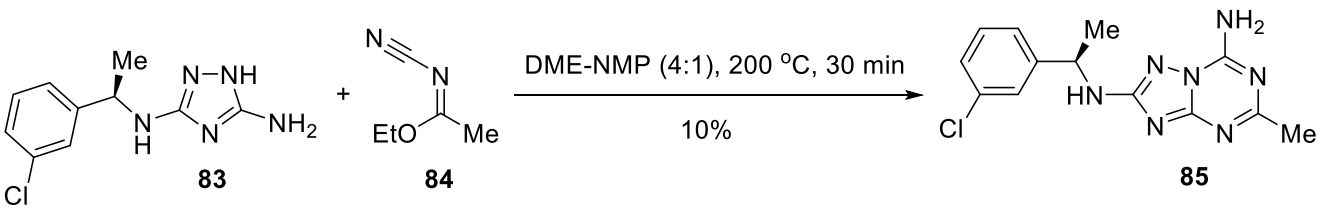
$IC_{50}$ ($hA_{2a}$) = 912 nM
$EC_{50}$ ($hD_2$) = 88 nM



**82**

$K_i$ ($hA_1$) > 5000 nM
$K_i$ ($hA_{2a}$) = 7.32 nM
$K_i$ ($rA_{2a}$) = 2.07 nM
$IC_{50}$ ($hA_{2b}$) > 5000 nM
$K_i$ ($hA_3$) > 5000 nM
$K_i$ ($rD_2$) > 5000 nM

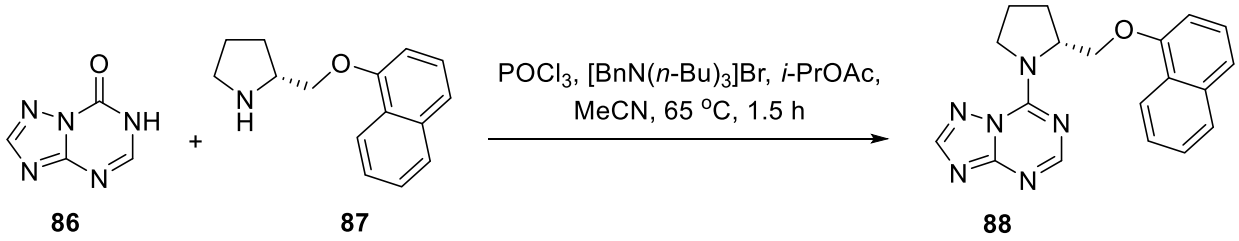
Figure 3




**83** **84** **85**

Scheme 26

In the Merck's program on the development of novel reversible methionine aminopeptidase-2 (MetAP-2) inhibitors, 1,2,4-triazolo[1,5-*a*][1,3,5]triazine **88** was synthesized by the amination of 1,2,4-triazolo[1,5-*a*][1,3,5]triazin-7(6*H*)-one (**86**) with pyrrolidine **87** (Scheme 27).[65] Even though **88** was found to be active against MetAP-2 ($IC_{50}$ = 1.8 μM), it was less effective in the inhibition of this enzyme than its purine and 1,2,4-triazolo[1,5-*a*]pyrinidine analogues.




**86** **87** **88**

Scheme 27

Thymidine phosphorylase is known as one of proangiogenic factors overexpressed in many solid tumors and therefore inhibitors of this enzyme might find an application as anticancer agents.[63] In the search for potential thymidine phosphorylase inhibitors, one series of 1,2,4-triazolo[1,5-*a*][1,3,5]triazines *viz*. **24** was identified to be active.[16-19] The substitution pattern on the triazine ring *viz*. a carbonyl group in the position 7 and a thiocarbonyl group in the position 5 were critical for the activity. The attempts to swap positions of these groups or replace the thiocarbonyl with another carbonyl group resulted in the activity loss.[17] The *S*-alkylation also produced significantly less active compounds.[19] Some improvements in the activity were achieved when a phenyl ring was introduced to the position 2 (*cf.* **24a** and **24b**) and the activity tolerated various substituents in this position.[17] The extensive screening of 2-aryl-substituted library of **24** identified **24c** as one of the most potent compounds.[16] The enzyme kinetic data suggested a competitive mixed type (with respect to thymidine) mechanism of inhibition. The experiments using human breast adenocarcinoma MDA-MB-231 cells established that **24c** inhibited expression of angiogenesis markers: vascular endothelial growth factor (VEGF) and matrix metallopeptidase 9 (MMP-9).[16] The thymidine phosphorylase inhibitory properties were further improved in compound **24d** when a methylene linkage was introduced between the 1,2,4-triazolo[1,5-*a*][1,3,5]triazine ring and the dichlorophenyl group. Similarly to **24c**, the more flexible analogue **24d** was a competitive mixed type inhibitor of thymidine phosphorylase. However, significantly suppressing VEGF expression, **24d** only at very high concentrations affected the MMP-9 production.

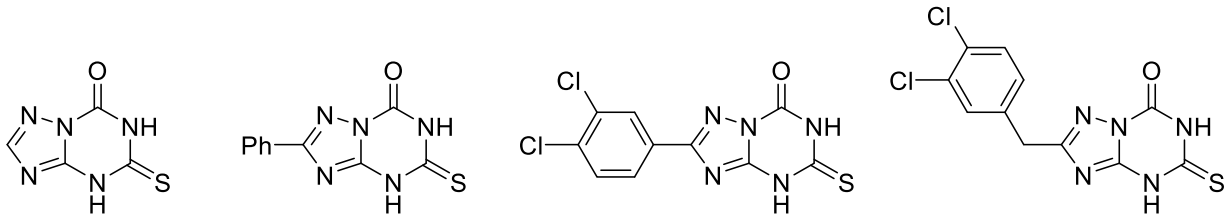



Figure 4

### 3.3. Compounds targeting other regulatory mechanisms

The screening for compounds disrupting the proteasome system in *Mycobacterium tuberculosis* using the STructural INTeractions by in-cell NMR (STINT-NMR) was developed and applied to a library of 1597 diverse drug-like compounds.[66] One of the three identified in the STINT-NMR screening compounds selectively targeting the proteasome degradation pathway was 6-methyl-1,2,4-triazolo[1,5-*a*][1,3,5]triazine-7(6*H*)-thione (**89**). Effectively disrupting interactions between mycobacterial ATPase (Mpa) and prokaryotic ubiquitin like protein (Pup) in cells, **89** was found to bind specifically to Mpa in sub-micromolar concentrations ($K_d$ = 0.4 μM).

Compound **90**, prepared by the alkylation of **24**, was listed together with numerous triazolopyrimidine analogues in the patent on inhibitors of fatty acid binding protein (FABP).[67] However, its affinity (expressed as $K_d$) to FABP4 reported in the patent was above 0.5 μM.

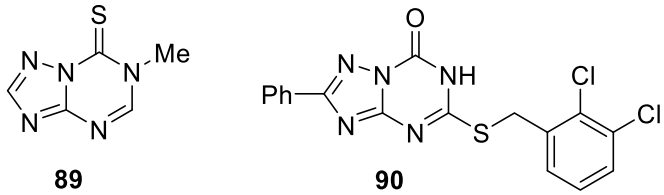
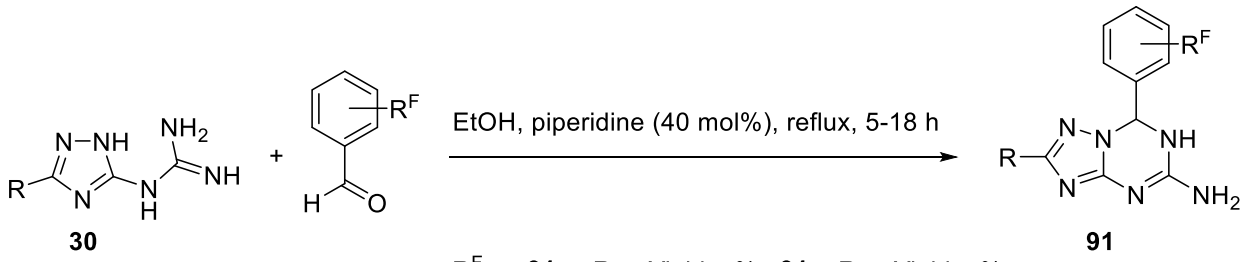



Figure 5

### 3.4. Anticancer agents

In the search for new anticancer agents, a series of fluorinated 1,2,4-triazolo[1,5-*a*][1,3,5]triazines **91** was prepared from 5-guanidino-1,2,4-triazoles **30** (Scheme 28).[68] The higher activity was found against MDA-MB-231 breast cancer cell and the most active compound in the series was **91f**. Further attempts to prepare structurally similar compounds with more metabolically stable fully aromatic 1,2,4-triazolo[1,5-*a*][1,3,5]triazine system but still retaining substitution pattern suitable for similar hydrogen bonding led to the design of compounds **36** (Scheme 29).[69] These compounds were synthesized from 1,2,4-triazolo[1,5-*a*][1,3,5]triazine **35** *via* nucleophilic displacement of the trichloromethyl group at the electron-deficient triazine ring of **35** by fluorinated benzylamines. The structures of the prepared

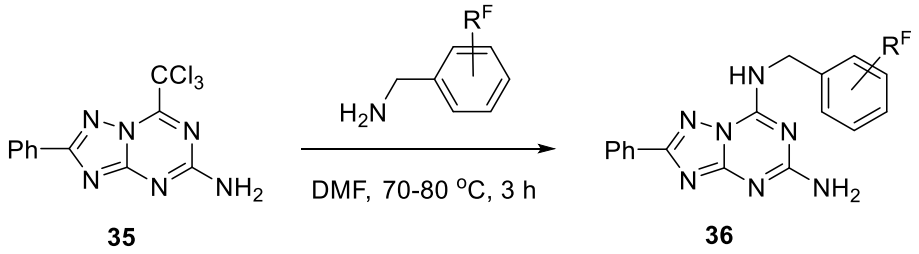
compounds **36** were confirmed using NMR spectroscopy and also by X-ray crystallography for the representative product **36c**.[70] Similarly to the series **91**, the most active in the series **35** against MDA-MB-231 breast cancer cells was the analogue **35f** with 4-trifluoromethyl substituent on the phenyl ring.[69] This compound (**35f**) was equally active against A549 lung cancer cells. Interestingly, being less potent in antiproliferative assay compound **35g** demonstrated good anti-angiogenic properties with an $EC_{50}$ value of 9.4 μM in the anti-angiogenesis tube formation assay.




**30** **91**

| $R^F$ | **91** | R | Yields, % | **91** | R | Yields, % |
|---|---|---|---|---|---|---|
| 2-F | **a** | 3-Py | 85 | **g** | 4-Py | 85 |
| 3-F | **b** | 3-Py | 87 | **h** | 4-Py | 87 |
| 4-F | **c** | 3-Py | 91 | **i** | 4-Py | 92 |
| 2-$CF_3$ | **d** | 3-Py | 87 | j | 4-Py | 86 |
| 3-$CF_3$ | **e** | 3-Py | 86 | **k** | 4-Py | 90 |
| 4-$CF_3$ | **f** | 3-Py | 93 | **l** | 4-Py | 88 |

Scheme 28




**35** **36**

| **36** | $R^F$ | Yields, % |
|---|---|---|
| **a** | 2-F | 85 |
| **b** | 3-F | 87 |
| **c** | 4-F | 88 |
| **d** | 2-$CF_3$ | 90 |
| **e** | 3-$CF_3$ | 92 |
| **f** | 4-$CF_3$ | 94 |
| **g** | 3,5-$(CF_3)_2$ | 93 |

Scheme 29

## 4. CONCLUSION

In the development of new methods for the synthesis of 1,2,4-triazolo[1,5-*a*][1,3,5]triazines, several efficient approaches, including multicomponent reactions, were designed. New effective reagents for the selective 1,3,5-triazine ring closure were introduced and successfully applied for the synthesis of 1,2,4-triazolo[1,5-*a*][1,3,5]triazines. Advancements in the area of biological activities of

1,2,4-triazolo[1,5-*a*][1,3,5]triazines have continued to focus primarily on the development of new adenosine receptor antagonists. Some interesting results were also obtained in the search of enzyme inhibitors and potential anticancer agents.

**ACKNOWLEDGEMENTS**

This work is supported by the Ministry of Higher Education, Malaysia under Fundamental Research Grant Scheme, grant number FRGS/1/2015/SG01/MUSM/03/1.

**REFERENCES**

1. F. P. L. Lim, A. V. Dolzhenko, and A. V. Dolzhenko, 'Development of new enzyme inhibitors: Purine bioisostere perspective,' in: *Pharmaceutical Formulation and Medicinal Chemistry: Mechanisms, Developments and Treatments*, ed. by B. Moore, Nova Science Publishers, Inc., 2016, pp. 107-130.
2. M. Knapp, C. Bellamacina, J. M. Murray, and D. E. Bussiere, *Curr. Top. Med. Chem.*, 2006, **6**, 1129.
3. V. L. Rusinov, V. N. Charushin, and O. N. Chupakhin, *Russ. Chem. Bull.*, 2018, **67**, 573.
4. F. P. L. Lim and A. V. Dolzhenko, *Eur. J. Med. Chem.*, 2014, **85**, 371.
5. A. V. Dolzhenko, A. V. Dolzhenko, and W. K. Chui, *Heterocycles*, 2006, **68**, 1723.
6. A. V. Dolzhenko, S. A. Kalinina, and D. V. Kalinin, *RSC Adv.*, 2013, **3**, 15850.
7. S. A. Kalinina, D. V. Kalinin, and A. V. Dolzhenko, *Tetrahedron Lett.*, 2013, **54**, 5537.
8. P. W. R. Caulkett, G. Jones, M. McPartin, N. D. Renshaw, S. K. Stewart, and B. Wright, *J. Chem. Soc., Perkin Trans. 1*, 1995, 801.
9. A. Y. Hassan, M. T. Sarg, A. H. Bayoumi, and M. A. El-Deeb, *J. Heterocycl. Chem.*, 2018, **55**, 1450.
10. F. Akahoshi, S. Takeda, T. Okada, M. Kajii, H. Nishimura, M. Sugiura, Y. Inoue, C. Fukaya, Y. Naito, T. Imagawa, and N. Nakamura, *J. Med. Chem.*, 1998, **41**, 2985.
11. A. V. Dolzhenko, A. V. Dolzhenko, and W.-K. Chui, *Heterocycles*, 2007, **71**, 429.
12. J. Ma, G. Cheng, X. Ju, Z. Yi, S. Zhu, Z. Zhang, and H. Yang, *Dalton Trans.*, 2018, **47**, 14483.
13. M. Khankischpur, F. K. Hansen, and D. Geffken, *Synthesis*, 2010, 1645.
14. L. L. Zamigailo, O. N. Petrova, M. G. Shirobokova, and V. V. Lipson, *Russ. J. Org. Chem.*, 2013, **49**, 288.
15. B. A. Demidchuk, V. S. Brovarets, A. N. Chernega, J. A. K. Howard, A. N. Vasilenko, A. V. Turov, and B. S. Drach, *Russ. J. Gen. Chem.*, 2007, **77**, 474.
16. H. Bera, B. J. Tan, L. Sun, A. V. Dolzhenko, W.-K. Chui, and G. N. C. Chiu, *Eur. J. Med. Chem.*, 2013, **67**, 325.
17. H. Bera, A. V. Dolzhenko, L. Sun, S. Dutta Gupta, and W.-K. Chui, *Chem. Biol. Drug Des.*, 2013, **82**,

351.
18. H. Bera, W.-K. Chui, S. Dutta Gupta, A. V. Dolzhenko, and L. Sun, *Med. Chem. Res.*, 2013, **22**, 6010.
19. H. Bera, M. H. Lee, L. Sun, A. V. Dolzhenko, and W. K. Chui, *Bioorg. Chem.*, 2013, **50**, 34.
20. D. V. Kalinin, S. A. Kalinina, and A. V. Dolzhenko, *Heterocycles*, 2012, **85**, 2515.
21. D. V. Kalinin, S. A. Kalinina, and A. V. Dolzhenko, *Heterocycles*, 2013, **87**, 147.
22. A. V. Dolzhenko, A. V. Dolzhenko, and W.-K. Chui, *Tetrahedron*, 2007, **63**, 12888.
23. A. V. Dolzhenko and B. Ya. Syropyatov, *Pat. RU* 2330854, 2008 (*Chem. Abstr.*, **149**, 246567).
24. A. V. Dolzhenko, G. K. Tan, L. L. Koh, A. V. Dolzhenko, and W. K. Chui, *Acta Crystallogr. Sect. E*, 2007, **63**, o2796.
25. A. V. Dolzhenko, G. K. Tan, L. L. Koh, A. V. Dolzhenko, and W. K. Chui, *Crystals*, 2011, **1**, 136.
26. A. V. Dolzhenko, G. K. Tan, L. L. Koh, A. V. Dolzhenko, and W. K. Chui, *Acta Crystallogr. Sect. E*, 2007, **63**, o2797.
27. A. V. Dolzhenko, G. Pastorin, A. V. Dolzhenko, and W. K. Chui, *Tetrahedron Lett.*, 2008, **49**, 7180.
28. A. V. Dolzhenko, G. K. Tan, L. L. Koh, S. F. Woo, and W. K. Chui, *Acta Crystallogr. Sect. E*, 2008, **64**, o2021.
29. M. Wang, Y. Meng, W. Wei, J. Wu, W. Yu, and J. Chang, *Adv. Synth. Catal.*, 2018, **360**, 86.
30. G. L. Hoang, K. S. Halskov, and J. A. Ellman, *J. Org. Chem.*, 2018, **83**, 9522.
31. V. V. Bakharev, V. E. Parfenov, I. V. Ul'yankina, A. V. Zavodskaya, E. V. Selezneva, A. A. Gidaspov, O. S. Eltsov, and P. A. Slepukhin, *Tetrahedron*, 2014, **70**, 6825.
32. I. V. Ul'yankina, A. V. Zavodskaya, V. E. Parfenov, A. A. Gidaspov, A. K. Shiryaev, E. V. Selezneva, and V. V. Bakharev, *Chem. Heterocycl. Compd.*, 2016, **52**, 1054.
33. V. V. Bakharev, V. E. Parfenov, I. V. Ul'yankina, A. V. Zavodskaya, A. A. Gidaspov, and P. A. Slepukhin, *Chem. Heterocycl. Compd.*, 2015, **51**, 1014.
34. A. V. Zavodskaya, V. V. Bakharev, V. E. Parfenov, A. A. Gidaspov, P. A. Slepukhin, M. L. Isenov, and O. S. Eltsov, *Tetrahedron Lett.*, 2015, **56**, 1103.
35. S. Federico, A. Ciancetta, N. Porta, S. Redenti, G. Pastorin, B. Cacciari, K. N. Klotz, S. Moro, and G. Spalluto, *Eur. J. Med. Chem.*, 2016, **108**, 529.
36. V. A. Tartakovsky, A. E. Frumkin, A. M. Churakov, and Y. A. Strelenko, *Russ. Chem. Bull.*, 2005, **54**, 719.
37. R. Huisgen, H. J. Sturm, and M. Seidel, *Chem. Ber.*, 1961, **94**, 1555.
38. T. Rieth, N. Roeder, M. Lehmann, and H. Detert, *Chem. Eur. J.*, 2018, **24**, 93.
39. J. W. Fronabarger, R. D. Chapman, and R. D. Gilardi, *Tetrahedron Lett.*, 2006, **47**, 7707.
40. M. T. Armentero, A. Pinna, S. Ferré, J. L. Lanciego, C. E. Müller, and R. Franco, *Pharmacol. Ther.*,

2011, **132**, 280.

41. P. Jenner, *Int. Rev. Neurobiol*., 2014, **119**, 71.
42. M. Jorg, M. Agostino, E. Yuriev, F. S. Mak, N. D. Miller, J. M. White, P. J. Scammells, and B. Capuano, *Struct. Chem.*, 2013, **24**, 1241.
43. P. W. R. Caulkett, G. Jones, M. G. Collis, and S. M. Poucher, *Pat. EP* 459702, 1991 (*Chem. Abstr.*, **117**, 171476).
44. V.-P. Jaakola, M. T. Griffith, M. A. Hanson, V. Cherezov, E. Y. T. Chien, J. R. Lane, A. P. Ijzerman, and R. C. Stevens, *Science*, 2008, **322**, 1211.
45. P. Rucktooa, R. K. Y. Cheng, E. Segala, T. Geng, J. C. Errey, G. A. Brown, R. M. Cooke, F. H. Marshall, and A. S. Dore, *Sci. Rep*., 2018, **8**, 41.
46. M. T. Eddy, M. Y. Lee, Z. G. Gao, K. L. White, T. Didenko, R. Horst, M. Audet, P. Stanczak, K. M. McClary, G. W. Han, K. A. Jacobson, R. C. Stevens, and K. Wuthrich, *Cell*, 2018, **172**, 68.
47. T. Weinert, N. Olieric, R. Cheng, S. Brunle, D. James, D. Ozerov, D. Gashi, L. Vera, M. Marsh, K. Jaeger, F. Dworkowski, E. Panepucci, S. Basu, P. Skopintsev, A. S. Dore, T. Geng, R. M. Cooke, M. Liang, A. E. Prota, V. Panneels, P. Nogly, U. Ermler, G. Schertler, M. Hennig, M. O. Steinmetz, M. Wang, and J. Standfuss, *Nat. Commun*., 2017, **8**, 542.
48. J. M. Martin-Garcia, C. E. Conrad, G. Nelson, N. Stander, N. A. Zatsepin, J. Zook, L. Zhu, J. Geiger, E. Chun, D. Kissick, M. C. Hilgart, C. Ogata, A. Ishchenko, N. Nagaratnam, S. Roy-Chowdhury, J. Coe, G. Subramanian, A. Schaffer, D. James, G. Ketwala, N. Venugopalan, S. Xu, S. Corcoran, D. Ferguson, U. Weierstall, J. C. H. Spence, V. Cherezov, P. Fromme, R. F. Fischetti, and W. Liu, *IUCrJ*, 2017, **4**, 439.
49. I. Melnikov, V. Polovinkin, K. Kovalev, I. Gushchin, M. Shevtsov, V. Shevchenko, A. Mishin, A. Alekseev, F. Rodriguez-Valera, V. I. Borshchevskiy, V. Cherezov, G. A. Leonard, V. I. Gordeliy, and A. Popov, *Sci. Adv*., 2017, **3**, e1602952.
50. E. Segala, D. Guo, R. K. Cheng, A. Bortolato, F. Deflorian, A. S. Dore, J. C. Errey, L. H. Heitman, A. P. IJzerman, F. H. Marshall, and R. M. Cooke, *J. Med. Chem*., 2016, **59**, 6470.
51. A. Batyuk, L. Galli, A. Ishchenko, G. W. Han, C. Gati, P. A. Popov, M. Y. Lee, B. Stauch, T. A. White, A. Barty, A. Aquila, M. S. Hunter, M. Liang, S. Boutet, M. Pu, Z. J. Liu, G. Nelson, D. James, C. Li, Y. Zhao, J. C. Spence, W. Liu, P. Fromme, V. Katritch, U. Weierstall, R. C. Stevens, and V. Cherezov, *Sci. Adv*., 2016, **2**, e1600292.
52. W. Liu, E. Chun, A. A. Thompson, P. Chubukov, F. Xu, V. Katritch, G. W. Han, C. B. Roth, L. H. Heitman, A. P. IJzerman, V. Cherezov, and R. C. Stevens, *Science*, 2012, **337**, 232.
53. T. Hino, T. Arakawa, H. Iwanari, T. Yurugi-Kobayashi, C. Ikeda-Suno, Y. Nakada-Nakura, O. Kusano-Arai, S. Weyand, T. Shimamura, N. Nomura, A. D. Cameron, T. Kobayashi, T. Hamakubo,

S. Iwata, and T. Murata, *Nature*, 2012, **482**, 237.

54. A. S. Doré, N. Robertson, J. C. Errey, I. Ng, K. Hollenstein, B. Tehan, E. Hurrell, K. Bennett, M. Congreve, F. Magnani, C. G. Tate, M. Weir, and F. H. Marshall, *Structure*, 2011, **19**, 1283.
55. G. Pastorin, S. Federico, S. Paoletta, M. Corradino, F. Cateni, B. Cacciari, K.-N. Klotz, Z.-G. Gao, K. A. Jacobson, G. Spalluto, and S. Moro, *Bioorg. Med. Chem.*, 2010, **18**, 2524.
56. S. Federico, S. Paoletta, S.-L. Cheong, G. Pastorin, B. Cacciari, S. Stragliotto, K. N. Klotz, J. Siegel, Z.-G. Gao, K. A. Jacobson, S. Moro, and G. Spalluto, *J. Med. Chem.*, 2011, **54**, 877.
57. D. Guo, L. Xia, J. P. D. van Veldhoven, M. Hazeu, T. Mocking, J. Brussee, A. P. IJzerman, and L. H. Heitman, *ChemMedChem*, 2014, **9**, 752.
58. X. Yang, G. Dong, T. J. M. Michiels, E. B. Lenselink, L. Heitman, J. Louvel, and A. P. Ijzerman, *Purinergic Signal.*, 2017, **13**, 191.
59. X. Yang, T. J. M. Michiels, C. de Jong, M. Soethoudt, N. Dekker, E. Gordon, M. van der Stelt, L. H. Heitman, D. van der Es, and A. P. IJzerman, *J. Med. Chem.*, 2018, **61**, 7892.
60. M. Jorg, J. Shonberg, F. S. Mak, N. D. Miller, E. Yuriev, P. J. Scammells, and B. Capuano, *Bioorg. Med. Chem. Lett.*, 2013, **23**, 3427.
61. M. Jorg, L. T. May, F. S. Mak, K. C. K. Lee, N. D. Miller, P. J. Scammells, and B. Capuano, *J. Med. Chem.*, 2015, **58**, 718.
62. A. Dalpiaz, B. Cacciari, C. B. Vicentini, F. Bortolotti, G. Spalluto, S. Federico, B. Pavan, F. Vincenzi, P. A. Borea, and K. Varani, *Mol. Pharm.*, 2012, **9**, 591.
63. Y. Y. Elamin, S. Rafee, N. Osman, K. J. O'Byrne, and K. Gately, *Cancer Microenviron.*, 2015, **9**, 33.
64. R. J. Moreau, C. K. Skepper, B. A. Appleton, A. Blechschmidt, C. J. Balibar, B. M. Benton, J. E. Drumm, B. Y. Feng, M. Geng, C. Li, M. K. Lindvall, A. Lingel, Y. Lu, M. Mamo, W. Mergo, V. Polyakov, T. M. Smith, K. Takeoka, K. Uehara, L. Wang, J.-R. Wei, A. H. Weiss, L. Xie, W. Xu, Q. Zhang, and J. de Vicente, *J. Med. Chem.*, 2018, **61**, 3309.
65. T. Heinrich, H.-P. Buchstaller, B. Cezanne, F. Rohdich, J. Bomke, M. Friese-Hamim, M. Krier, T. Knoechel, D. Musil, B. Leuthner, and F. Zenke, *Bioorg. Med. Chem. Lett.*, 2017, **27**, 551.
66. C. M. De Mott, R. Girardin, J. Cobbert, S. Reverdatto, D. S. Burz, K. McDonough, and A. Shekhtman, *ACS Chem. Biol.*, 2018, **13**, 733.
67. G. W. Shipps, Jr., C. C. Cheng, X. Huang, A. A. Achab, P. Orth, J. H. Voigt, and K. A. Soucy, *Pat. WO* 2010056631, 2010 (*Chem. Abstr.*, **152**, 592045).
68. A. V. Dolzhenko, B. J. Tan, A. V. Dolzhenko, G. N. C. Chiu, and W. K. Chui, *J. Fluorine Chem.*, 2008, **129**, 429.
69. A. V. Dolzhenko, B. J. Tan, G. N. C. Chiu, W. K. Chui, and A. V. Dolzhenko, *J. Fluorine Chem.*,

2015, **175**, 68.

70. A. V. Dolzhenko, G. K. Tan, L. L. Koh, A. V. Dolzhenko, and W. K. Chui, *Acta Crystallogr. Sect. E*, 2011, **67**, o1183.

**Kow Koon Kee** was graduated from the University Putra Malaysia, Malaysia with a BSc (Hons) Chemistry (1st class) degree. She received the full scholarship for her postgraduate study from Monash University Malaysia, where she is perusing now her PhD under supervision of Dr. Anton Dolzhenko. Her current research interests include medicinal chemistry, drug discovery and cancer biology.

**Anton V. Dolzhenko** received his degree (with Distinction) in Pharmacy in 2000 and then a PhD degree in Medicinal Chemistry in 2004, both from the Perm State Pharmaceutical Academy (Russia). After that he joined the Pharmacy Department of the National University of Singapore and worked there for 6 years before he moved to the School of Pharmacy, Curtin University (Australia) in 2010 and continued his academic career as a Senior Lecturer. In 2013, he joined the School of Pharmacy, Monash University Malaysia, where he is working now as an Associate Professor, the Director of the NMR Campus Infrastructure Platform, and the Deputy Head (Research) of the School. His current research interests include synthetic and structural aspects of chemistry of nitrogen heterocycles. He has been actively working on the development of new synthetic methods for the preparation of potentially bioactive heterocyclic compounds.