REVIEW

Vision on Synthetic and Medicinal Facets of 1,2,4-Triazolo[3,4-*b*][1,3,4]thiadiazine Scaffold

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Abstract

The present review article strives to compile the latest synthetic approaches for the synthesis of triazolothiadiazine and its derivatives, along with their diverse pharmacological activities, viz. anticancer, antimicrobial, analgesic and anti-inflammatory, antioxidant, antiviral, enzyme inhibitors (carbonic anhydrase inhibitors, cholinesterase inhibitors, alkaline phosphatase inhibitors, anti-lipase activity, and aromatase inhibitors) and antitubercular agents. The review focuses particularly on the structure–activity relationship of biologically important 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines, which have profound importance in drug design, discovery and development. In silico pharmacokinetic and molecular modeling studies have also been summarized. It is hoped that this review article will be of help to researchers engaged in the development of new biologically active entities for the rational design and development of new target-oriented 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine-based drugs for the treatment of multifunctional diseases.
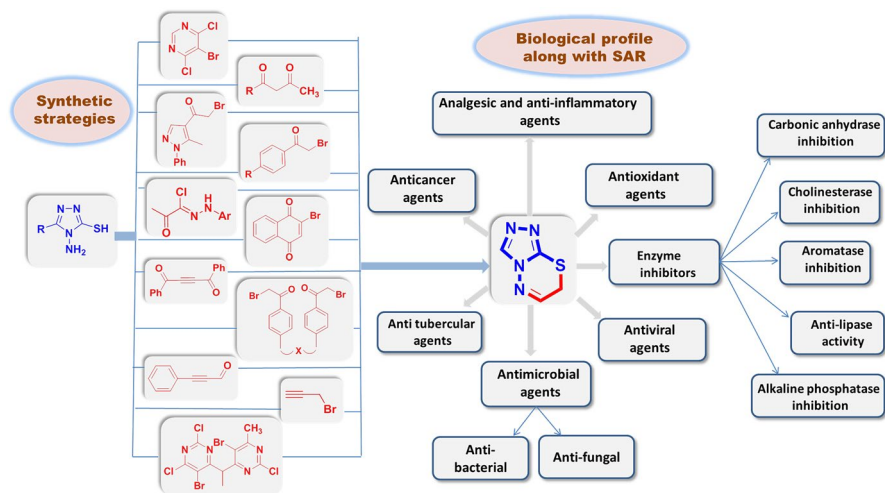
Extended author information available on the last page of the article

Graphical Abstract

1 Introduction

Heterocyclic compounds are of prime importance due to their extensive therapeutic uses [1]. One of the outstanding structural features innate to heterocycles, which continues to be exploited to great advantage by the drug industry, lies in their ability to manifest substituents around a core scaffold in defined three-dimensional (3D) representations. Among the collection of heterocycles, triazolothiadiazine, a hybrid nucleus made by fusion of two pharmaceutically active moieties, i.e., triazole and thiadiazine, is one of the most essential nuclei owing to their wide range of applications as synthetic intermediates and promising pharmaceuticals. The ability of hydrogen bond accepting and donating characteristics makes this core a much précised pharmacophore with a bioactive profile, as it can make specific interactions with different target receptors. On the basis of fusion permutations of a five-membered triazole ring with a six-membered thiadiazine ring, four isomeric structural variants of triazolothiadiazine, namely 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines (**I**), 1,2,4-triazolo[5,1-*b*][1,3,5]thiadiazines (**II**), 1,2,4-triazolo[1,5-*c*][1,3,5]thiadiazines (**III**), and 1,2,3-triazolo[5,1-*b*][1,3,4]thiadiazines (**IV**) may exist (Fig. 1).

As a class of privileged pharmacophores, the chemistry and synthesis of compounds possessing a 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine nucleus has attracted enormous attention. This review is focused on the synthetic protocols and biological potential, including structure–activity relationship (SAR), in silico
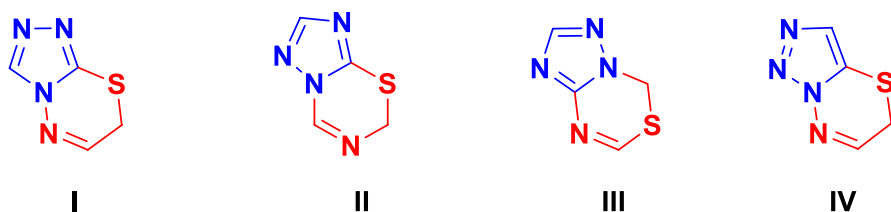
Fig. 1 Different possible isomers of triazolothiadiazine

pharmacokinetics, molecular modeling and mechanistic studies of 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines since 2010.

2 Synthetic Approaches to the 1,2,4-Triazolo[3,4-*b*][1,3,4]thiadiazine Scaffold

The literature is enriched with several approaches for synthesis of the 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine nucleus. Synthetic design for 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine involves primarily four routes: **a**, **b**, **c** and **d** (Fig. 2). Route **a** involves the acid-catalyzed cyclization of 2-hydrazinyl-6*H*-1,3,4-thiadiazine with ortho esters, thereby annulation of the triazole ring on thiadiazole [2]. However, this method has limited substrate scope. Route **b** involves the cyclocondensation of 4-amino-3-mercaptotriazoles with various bielectrophiles such as α-bromopropenone [3], phenacyl bromides [4–8], α-tosyloxy ketones [9], ethyl bromoacetate [10], hydrazonoyl halides [11], α-chloroacetylacetone [12] and propargyl bromide in presence of heteropolyacids [13]
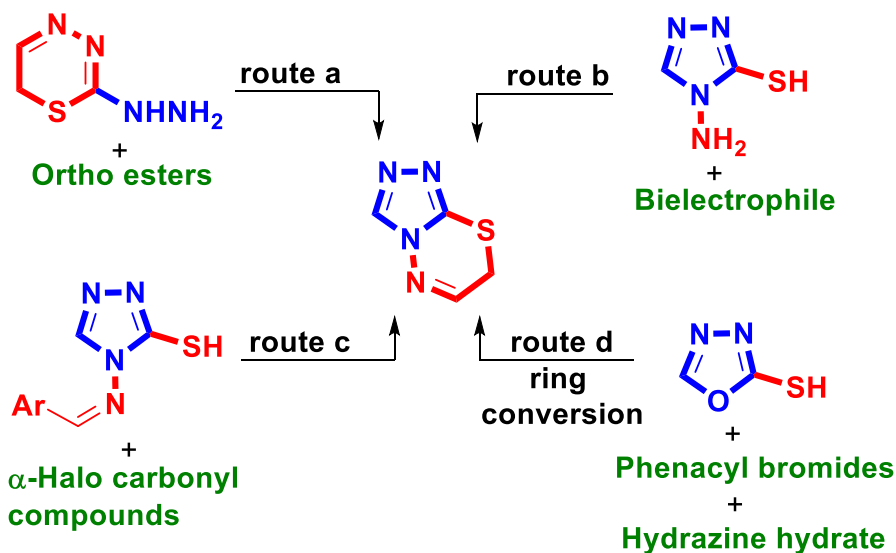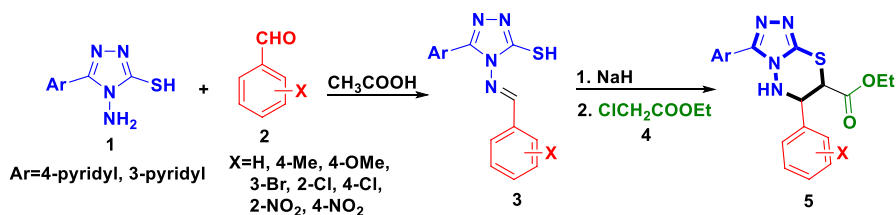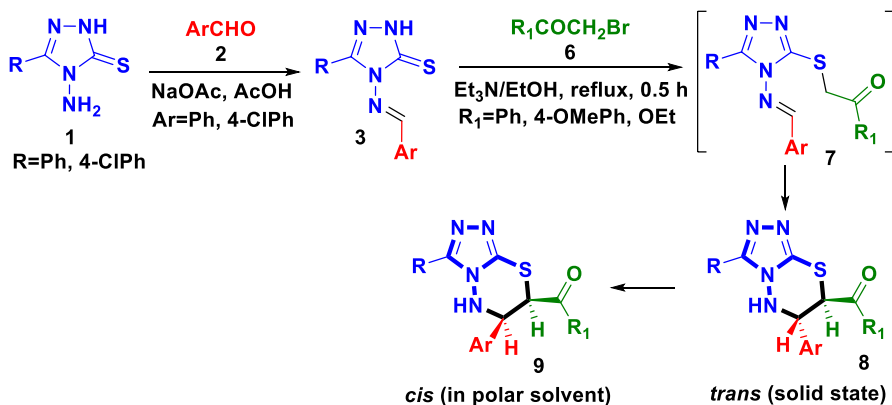

Fig. 2 Synthetic approaches for the preparation of 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine

Scheme 1 Synthesis of 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine derivatives


Scheme 2 Synthesis of 6,7-dihydro-5*H*-[1,2,4]triazolo[3,4-*b*]1,3,4]thiadiazine derivatives

under conventional heating conditions to access the triazolothiadiazine scaffold. This can be credited to mercapto and amino groups as easily accessible nucleophilic centers in 4-amino-3-mercaptotriazole for the synthesis of condensed heterocyclic rings. Route **b** offers vast diversity in terms of substitution at 5-position of 4-amino-3-mercaptotriazole as well as choices of bielectrophiles; therefore, this route is the most common approach for the synthesis of 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines. Route **c** involves synthesis of triazolothiadiazine derivatives by intermolecular condensation of 3-mercapto-4-arylideneamino-1,2,4-triazoles and α-halo compounds with activated methylene group [14, 15]. Route **d** involves ring transformation of oxadiazole-3*H*-thione upon treatment with phenacyl bromide followed by reaction with hydrazine hydrate to furnish triazolothiadiazine ring [16, 17].

The present review encapsulates and highlights the development of synthetic protocols in the last decade (2010–2021) for the synthesis of 1,2,4-triazolo[3,4-*b*][1,3,4] thiadiazines as illustrated in Schemes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33 and 34.

2.1 Synthetic Strategies Using *α*-Halocarbonyl Compounds

Foroughifar et al. [14] reported an efficient route for the synthesis of 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine derivatives (**5**) by treating Schiff's bases (**3**),
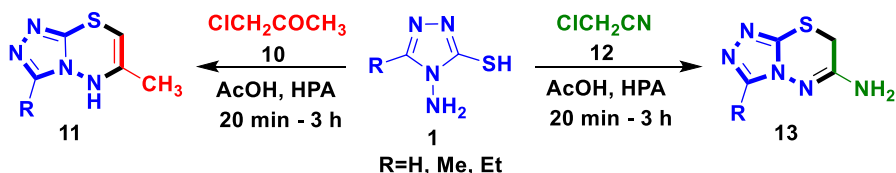
Scheme 3 Heteropolyacid catalyzed synthesis of [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine derivatives
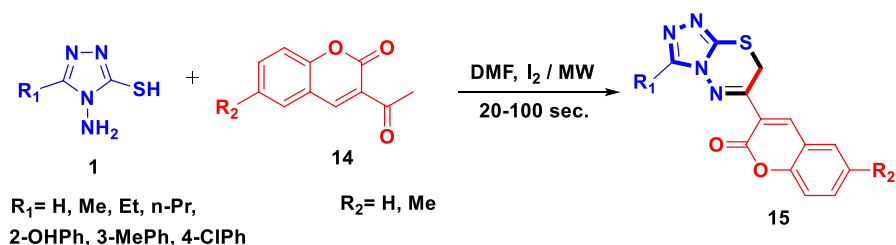
Scheme 4 Iodine mediated synthesis of [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines

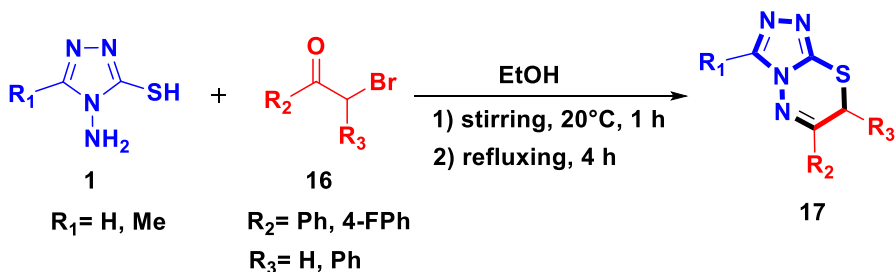**Scheme 5** Synthesis of trisubstituted 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine derivatives
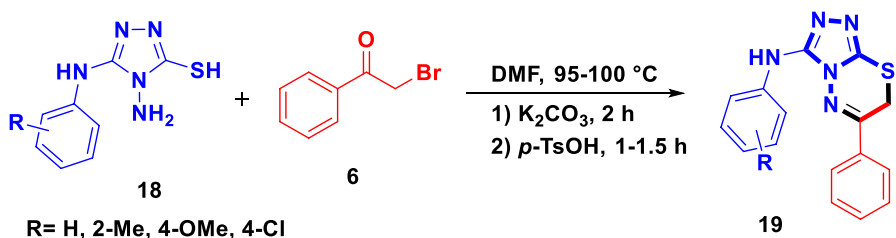
Scheme 6 Synthesis of 3-arylamino-6-phenyl[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine derivatives

obtained by reaction of 5-substituted 4-amino-1,2,4-triazole-3-thiols (**1**) with appropriate benzaldehydes (**2**) in acetic acid, with ethyl chloroacetate (**4**) in the presence of sodium hydride (Scheme 1).

Al-Etaibi et al. [15] used this method for stereoselective synthesis of 6,7-dihydro-5*H*-[1,2,4]triazolo[3,4-*b*]1,3,4]thiadiazine derivatives (**9**). Several
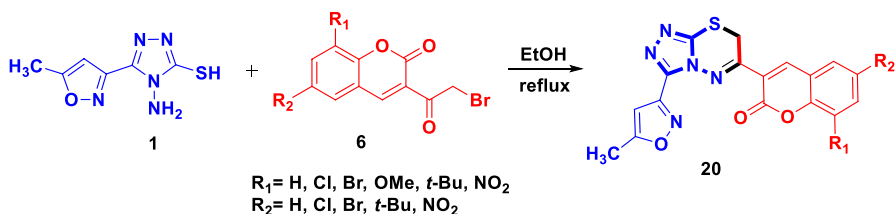
Scheme 7 Synthesis of coumarin substituted 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine derivatives
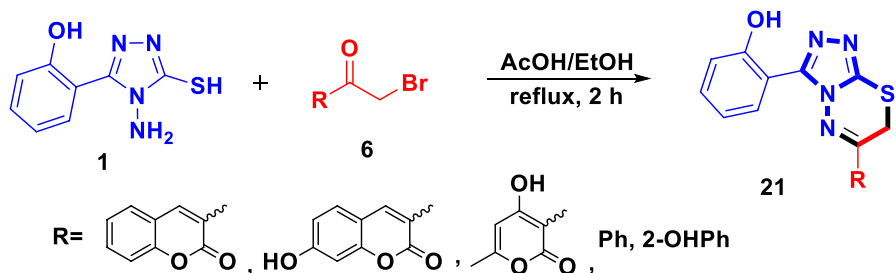
Scheme 8 Synthetic pathway for the formation of 1,2,4-triazolo[3,4-b][1,3,4]thiadiazine hybrids
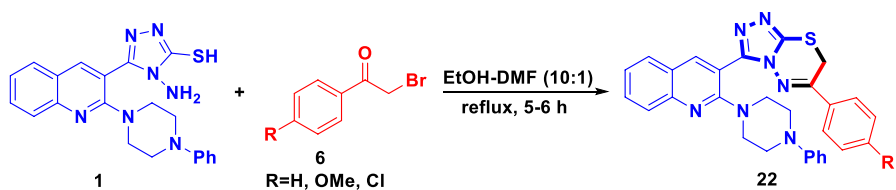
Scheme 9 Synthesis of 6-substituted-3-[2-(4-phenylpiperazin-1-yl)lquinolin-3-yl]-7*H*-[1,2,4]triazolo-thiadiazines
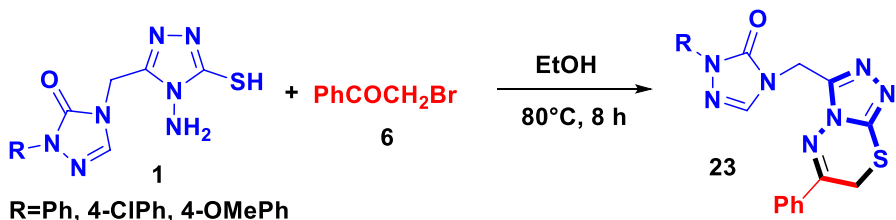
Scheme 10 Synthesis of 3,6-disubstituted[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine derivatives

4-arylideneamino-1,2,4-triazole-3(2*H*)-thiones (**3**) (generated by the reaction of 3-aryl-4-amino-5-mercapto-1,2,4-triazoles (**1**) with benzaldehydes (**2**) on condensation with ethyl bromoacetate or phenacyl bromide (**6**) in presence of triethylamine in refluxing ethanol gave trans-6,7-dihydro-5*H*-[1,2,4]triazolo[3,4-*b*]
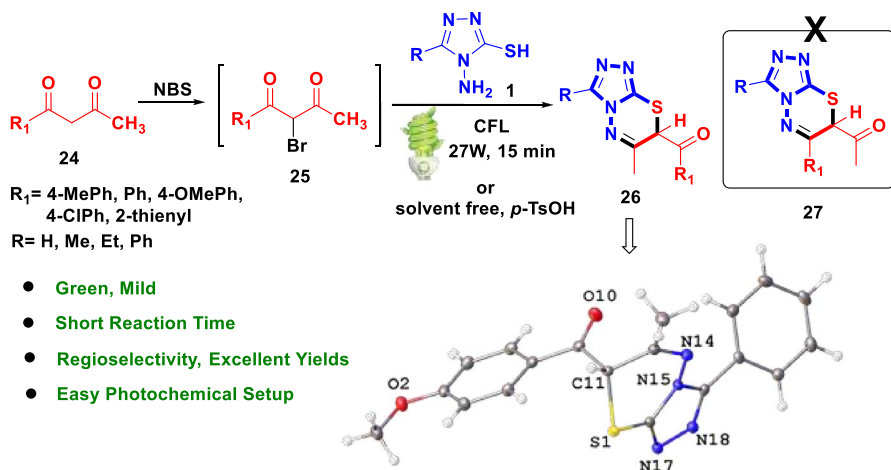
Scheme 11 Regioselective synthesis of 7-aroyl-6-methyl-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine analogs
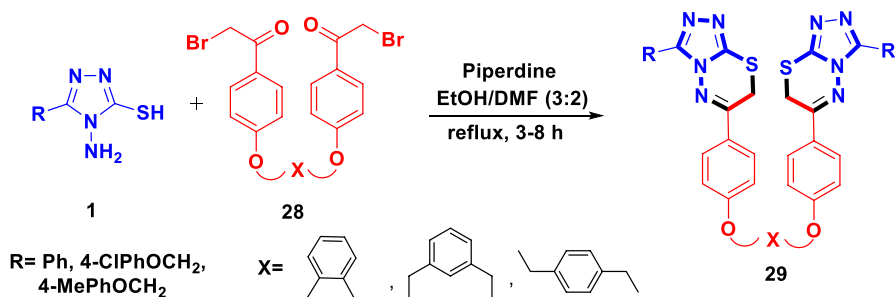
Scheme 12 Synthesis of bis(*s*-triazolo[3,4-*b*][1,3,4]thiadiazine) derivatives

[1,3,4]thiadiazines (**8**), which isomerized gradually in CDCl$_3$ and rapidly in DMSO-d$_6$ into corresponding cis-stereoisomers (**9**) (Scheme 2).

Motamedi et al. [18] developed a heteropolyacid (HPA)-catalyzed one-pot synthesis of [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine derivatives (**11**) and (**13**) in excellent yields via cyclocondensation reaction of 5-substituted 4-amino-1,2,4-triazole-3-thiols (**1**) with α-chloroacetone (**10**) or α-chloroacetonitrile (**12**) in refluxing acetic acid (Scheme 3).

Jakhar and Makrandi [19] described one-pot synthesis of [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine derivatives (**15**) by iodine-mediated condensation of 3-alkyl/aryl-4-amino-5-mercapto-1,2,4-triazoles (**1**) with 3-acetyl-6-substituted coumarin (**14**) in dimethylformamide (DMF) under microwave (MW) irradiation (Scheme 4). This protocol avoids the use of highly lachrymatory molecular bromine and its main advantage is good yields (78–92%) in a very short time.
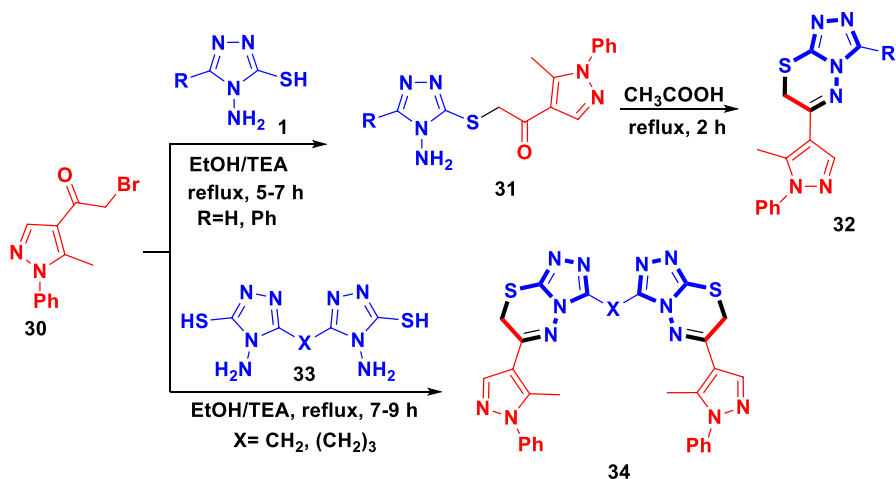
Scheme 13 Synthesis of pyrazole linked mono- and bis-1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine derivatives

X= Ph, 1,4-(CH$_2$)$_2$Ph, 1,4-(OCH$_2$)$_2$Ph, 1,2-(CH$_2$S)$_2$Ph
1,3-(CH$_2$S)$_2$Ph, 1,4-(CH$_2$S)$_2$Ph, 2,6-(CH$_2$S)$_2$py, 2,6-
(CH$_2$S)$_2$naphthalene, 9,10-(CH$_2$S)$_2$anthracene
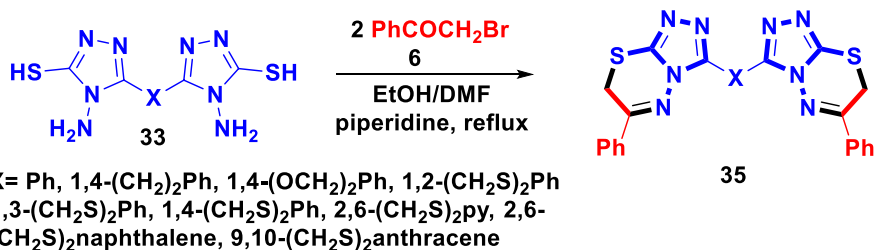
Scheme 14 Synthesis of bis(triazolo[3,4-*b*]thiadiazine) derivatives tagged to arene centres
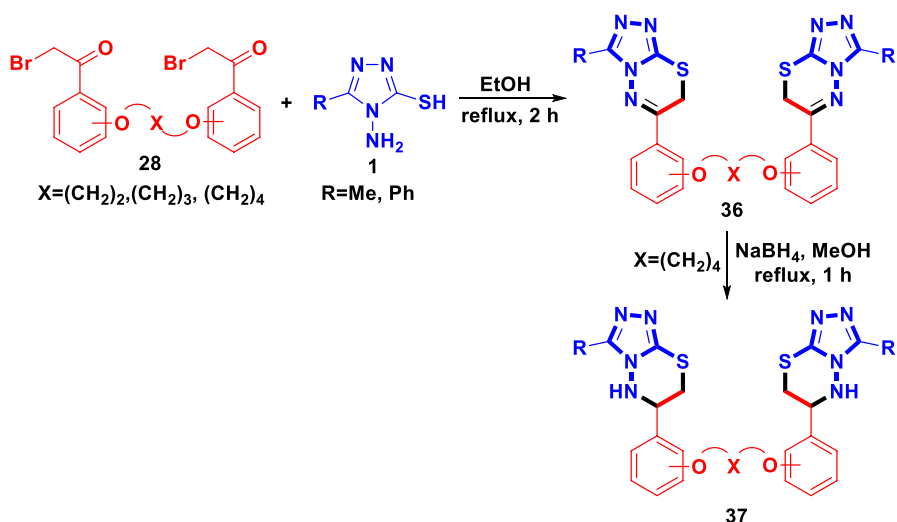
Scheme 15 Synthesis of bis(1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine) derivatives
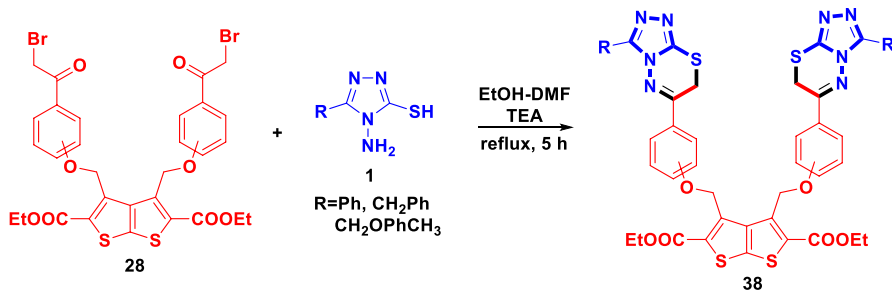
Scheme 16 Synthesis of bis(5,6-dihydro-s-triazolo[3,4-b]thiadiazines)
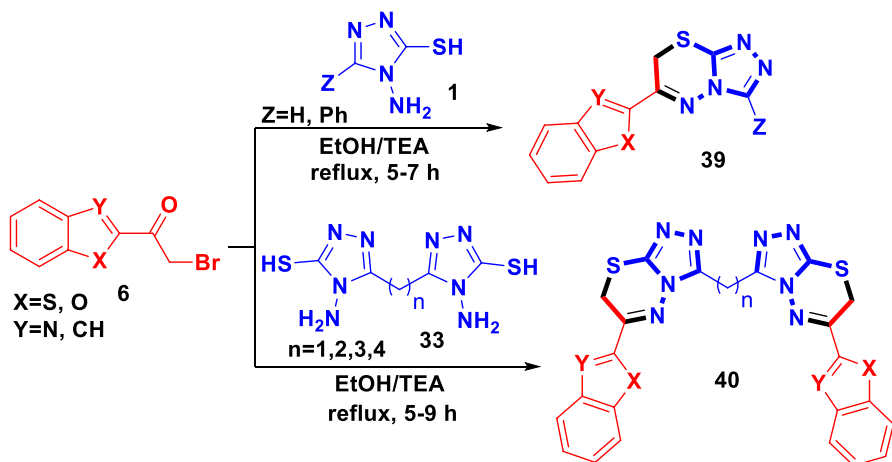
Scheme 17 Synthesis of benzofuran or benzo[d]thiazole linked mono- and bis-1,2,4-triazolo[3,4-b][1,3,4]thiadiazine derivatives
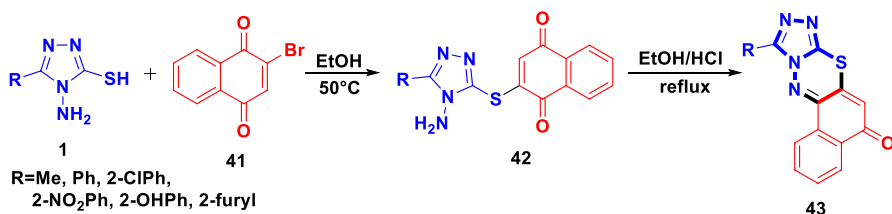
Scheme 18 Synthesis of naphthoquinone fused 1,2,4-triazolo[3,4-b][1,3,4]thiadiazine derivatives

Knak et al. [20] reported the synthesis of 3,6,7-trisubstituted 1,2,4-triazolo[3,4-b][1,3,4]thiadiazines (**17**) in excellent yields by stirring 4-amino-3-mercapto-l,2,4-triazoles (**1**) with 2-bromo-1,2-diphenylethan-1-ones (**16**) initially in ethanol at 20 °C and subsequently at refluxing for 4 h (Scheme 5).
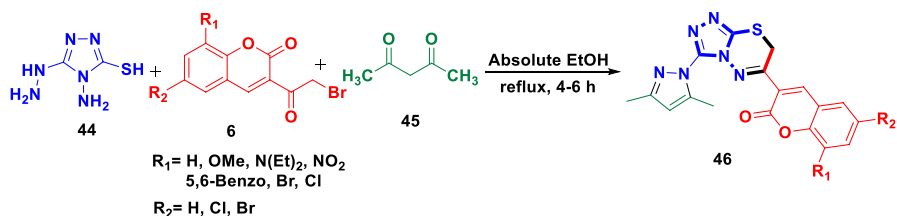
Scheme 19 Multicomponent assemblage for synthesis of 3-(pyrazolyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-6-yl)-2H-chromen-2-ones
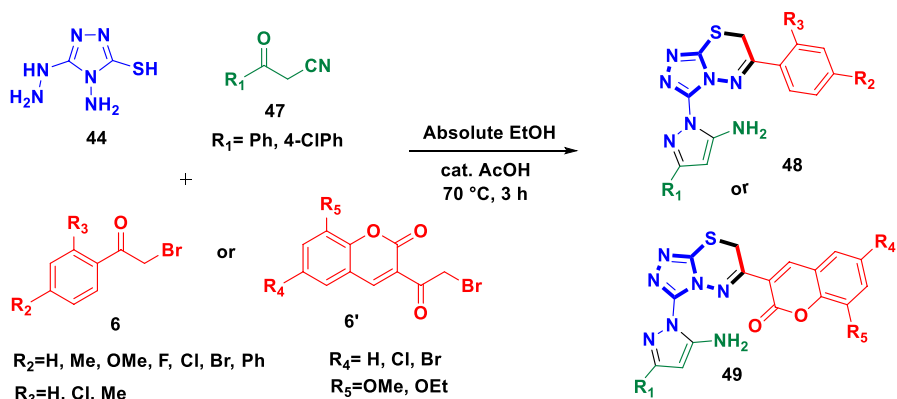
Scheme 20 Multicomponent synthesis of 3-aryl-1-(6-aryl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)-1H-pyrazol-5-amines and 3-(3-(5-amino-3-aryl-1H-pyrazol-1-yl)-7H-[1,2,4]triazolo[3,4-b] [1,3,4]thiadiazin-6-yl)-8-ethoxy-2H-chromen-2-ones
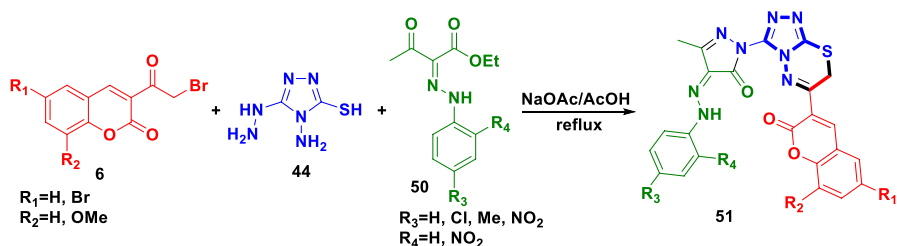
Scheme 21 Multicomponent synthesis of 4-(arylydrazono)-3-methyl-1-(6-(coumarin-3-yl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)-1H-pyrazol-5(4H)-ones

Marathe et al. [21] reported an efficient synthesis of 3-arylamino-6-phenyl[1,2,4]triazolo[3,4-b][1,3,4]thiadiazines derivatives (**19**) by heating 4-amino-3-arylamino-5-mercapto-1,2,4-triazoles (**18**) with α-bromoacetophenone (**6**) in dry DMF, initially in presence of potassium carbonate for 2 h, and subsequently with p-TsOH (Scheme 6).
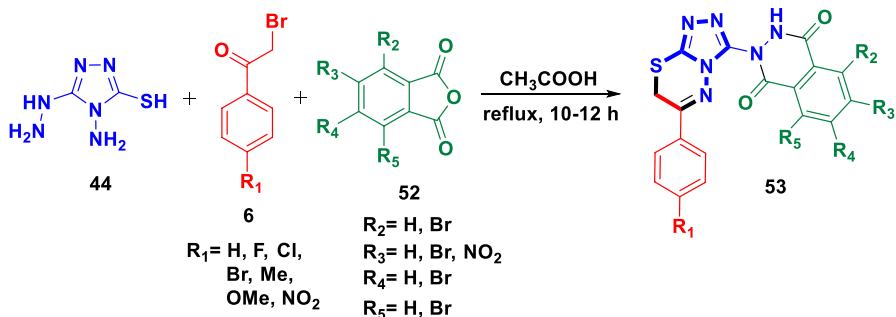
Scheme 22 Synthesis of phthalazinedione based 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines
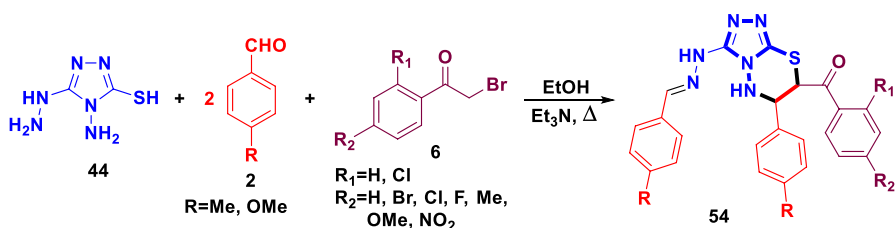
Scheme 23. Multicomponent synthesis of [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine derivatives
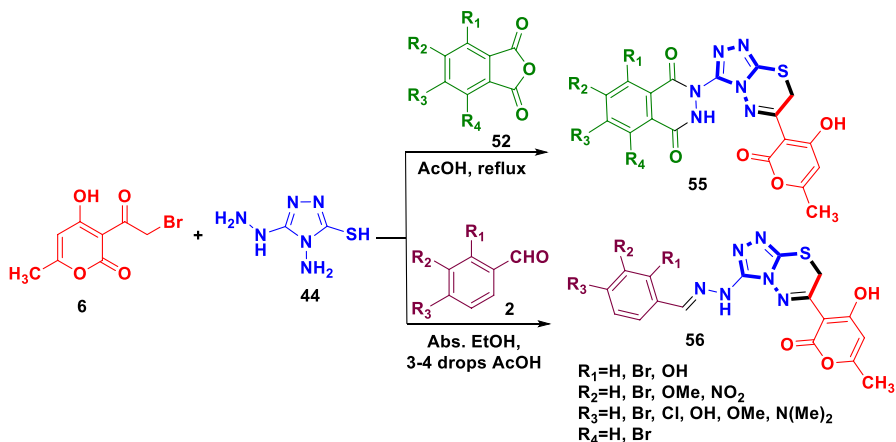
Scheme 24 One-pot multicomponent synthesis of substituted [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine derivatives

A literature survey revealed that manifestation of the coumarin nucleus in heterocycles is found to have broad pharmacological activities [22, 23]. Vaarla et al. [24] designed a series of coumarin substituted 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine derivatives (**20**) via cyclocondensation of 5-substituted
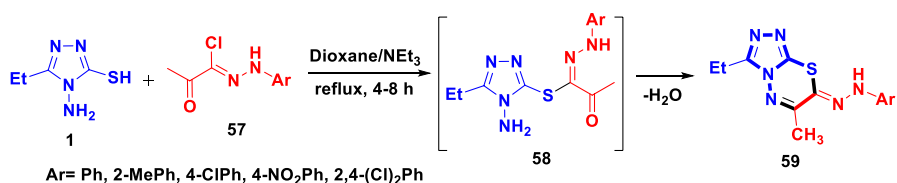
Scheme 25 Synthesis of 3-ethyl-6-methyl-7-(2-arylhydrazono)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine derivatives
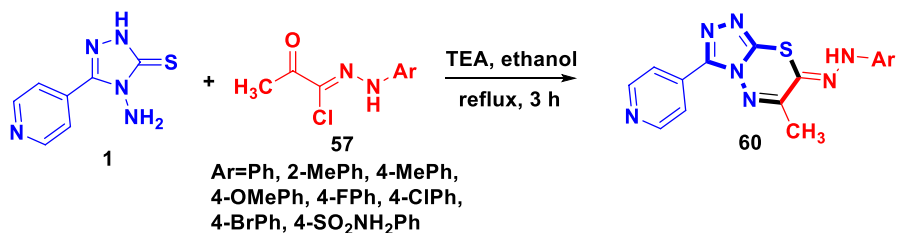
Scheme 26 Synthesis of (7Z)-7-[2-(aryl)hydrazinylidene]-6-methyl-3-(pyridine-4-yl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines
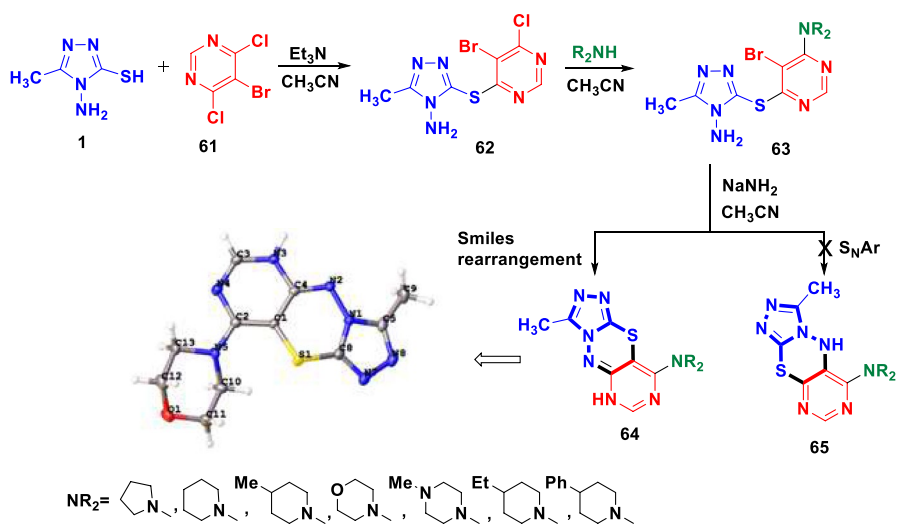
Scheme 27. Synthesis of pyrimidine clubbed 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine derivatives

4-amino-1,2,4-triazole-3-thiols (**1**) with differently substituted 3-(2-bromoacetyl) coumarins (**6**) in ethanol under reflux (Scheme 7). Substitution of coumarin with electron-withdrawing groups at R₁ and/or R₂ provided higher yields of products than electron rich groups.

Utilizing a conventional approach, Lechani et al. [25] synthesized [1,2,4] triazolo[3,4-*b*][1,3,4]thiadiazine hybrids bearing aryl-, pyran-2-one-, or coumarin
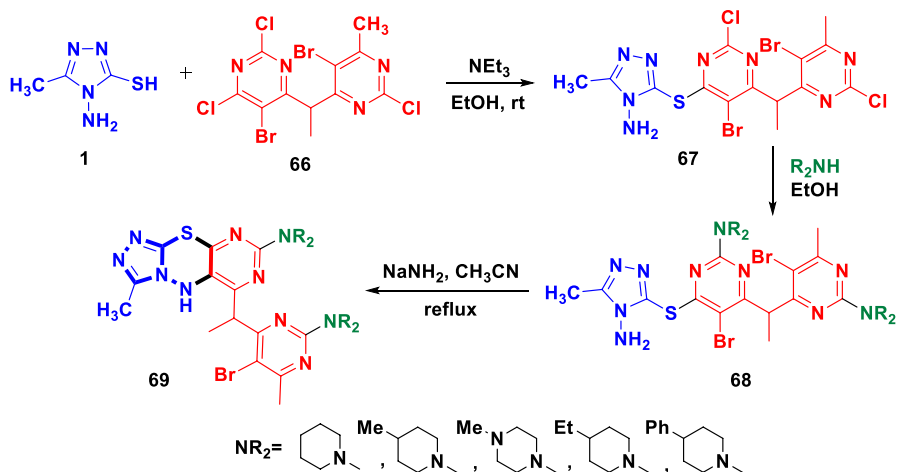
Scheme 28. Synthesis of pyrimido[5,4-*e*][1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine derivatives
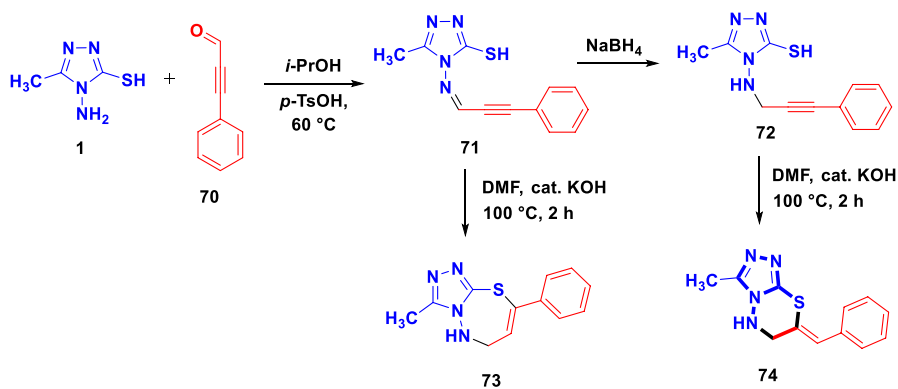
Scheme 29 Synthesis of 7-benzylidene-3-methyl-6,7-dihydro-5*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine
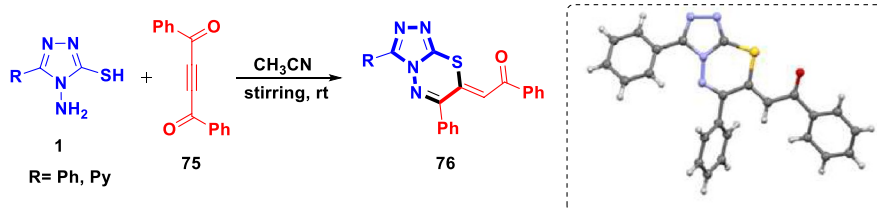
Scheme 30 Synthesis of highly functionalized 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines
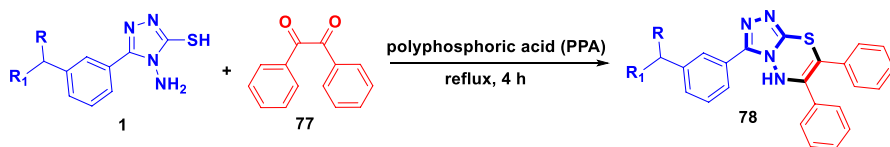
R=phthalimido, phthalimidomethyl,
2-phenyl-3-methyl-quinazolin-4-one
nicotinamido
R_1=H, Ph

Scheme 31 Synthesis of 5-substituted [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine) derivatives
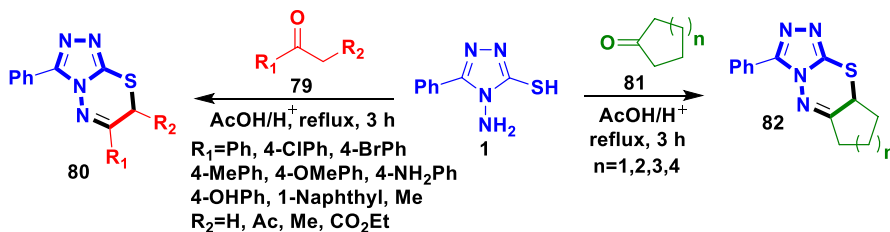
Scheme 32 One-step synthesis of triazolo[3,4-*b*][1,3,4]thiadiazines
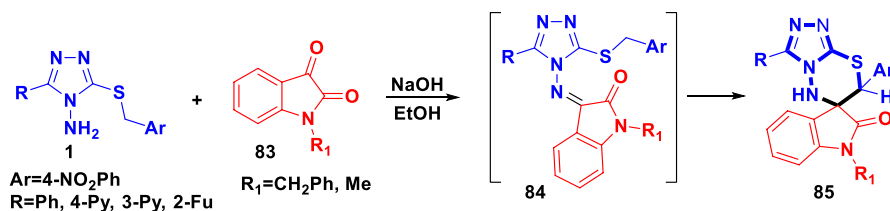
Scheme 33 Synthesis of spirocyclic triazolo[3,4-*b*][1,3,4]thiadiazine derivatives
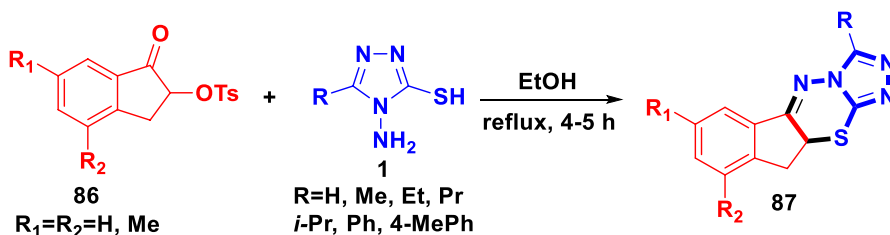
Scheme 34 Synthesis of 3-alkyl/aryl-7/9-methyl-10,10*a*-dihydroindeno[1,2-*e*][1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines

moieties (**21**) in moderate to good yields by acetic acid catalyzed cyclocondensation reaction of 2-(4-amino-5-mercapto-4*H*-[1,2,4]triazol-3-yl)phenol (**1**) with aromatic or heterocyclic α-bromoacetyl derivatives (**6**) in refluxing EtOH (Scheme 8). Simple reaction conditions, short reaction times, good yields and synthesis of highly

functionalized hybrids bearing oxygen-, nitrogen-, and sulfur-based heterocycles make this method potentially valuable.

Tang et al. [26] synthesized a new series of 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine derivatives (**22**) via reaction of 4-amino-5-[2-(2-piperazinyl-1-yl)quinolin-3-yl]-4*H*-1,2,4-triazole-3-thiol (**1**) with various phenacyl bromides (**6**) in refluxing ethanol/DMF mixture (10:1) (Scheme 9).

Kattimani et al. [27] successfully synthesized 3,6-disubstituted[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine derivatives (**23**) via reaction of phenacyl bromide (**6**) with various 4-(4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl)methyl)-2-aryl-2*H*-1,2,4-triazol-3(4*H*)-ones (**1**) in refluxing ethanol (Scheme 10).

In recent years, organic reactions under visible-light irradiation are grabbing intense attention as a powerful strategy involving the concept of 'Green Chemistry' to reduce hazardous ecological imprints of organic synthesis. In order to achieve a green protocol, Aggarwal et al. [28] developed first visible-light mediated regioselective synthesis of novel acyl derivatives of 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines **26** involving reaction of binucleophilic 4-amino-3-mercapto-1,2,4-triazoles (**1**) with in situ generated α-bromodiketones (**25**) from unsymmetrical β-diketones (**24**). In principle, reaction of unsymmetrical α-bromodiketones (**25**) (having two carbonyl carbon of different electrophilicity) with 4-amino-3-mercapto-1,2,4-triazoles (**1**) may lead to the formation of two regioisomers- 7-aroyl-6-methyl-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines (**26**) and 7-acetyl-6-aryl-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines (**27**). However, the reaction exhibited high level of regioselectivity yielding only (**26**), not the other possible isomer, 7-acetyl-6-aryl-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines (**27**). The structure of the regioisomer formed has been established unambiguously by heteronuclear 2D-NMR [(^{1}H–^{13}C) and (^{1}H–^{15}N) HMBC, (^{1}H–^{13}C) HMQC] spectroscopic and X-ray crystallographic studies. The substrate scope of the reaction was established by taking differently substituted 4-amino-3-mercapto-1,2,4-triazoles (**1**) and unsymmetrical diketones (**24**) under optimized conditions. All combinations resulted in exclusive formation of (**26**) with high regioselectivity. This rapid and efficient method involves use of simple and readily available starting materials, environment benign conditions, and provided excellent yields of the products (85–97%) in a fairly short time (Scheme 11). A solvent-free protocol using *p*-TsOH as a catalyst was also investigated to achieve the synthesis of (**26**); however, yields were comparatively lower (72–85%).

Bis-heterocycles with a flexible linker reported to possess a wide array of pharmaceutical and biological activities [29]. In this context, Ginidi et al. [30] introduced a novel series of bis(*s*-triazolo[3,4-*b*][1,3,4]thiadiazines) (**29**) tethered via phenoxymethyl spacers. Piperidine catalyzed cyclocondensation reaction of 4-amino-3-mercapto-1,2,4-triazole derivatives (**1**) with bis(α-bromoketones) (**28**) in EtOH-DMF mixture under reflux afforded corresponding bis(*s*-triazolo[3,4-*b*][1,3,4]thiadiazines) (**29**) in good yields (Scheme 12).

Synthesis of novel mono- and bis-1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines linked with pyrazole moiety (**32**) and (**34**) was described by Salem et al. [31]. The reaction of 4-amino-3-mercapto-1,2,4-triazole derivatives (**1**) with 2-bromo-1-(5-methyl-1-phenyl-1*H*-pyrazol-4-yl)ethanone (**30**) in refluxing ethanol in the presence of triethylamine as catalyst, afforded an intermediate (**31**), which underwent cyclization in

refluxing acetic acid to access pyrazolyl(5,6-dihydro-*s*-triazolo[3,4-*b*]thiadiazines) (**32**) in good yields (70–75%) (Scheme 13). Bis(6-pyrazolyl-*s*-triazolo[3,4-*b*][1,3,4] thiadiazines) linked to the alkyl spacer (**34**) were synthesized in one-step by cyclo-condensation of bis(4-amino-5-mercapto-1,2,4-triazol-3-yl)alkanes (**33**) with 2 equivalents of (**30**) in refluxing EtOH containing a few drops of triethylamine.

Alternatively, bis(triazolo[3,4-*b*]thiadiazine) derivatives (**35**) linked to arene centres via alkyl/ether/sulfanylmethylene insertions were prepared by treating bis(4-amino-5-mercapto-*s*-triazolo-3-y1) derivatives (**33**) with two equivalents of phenacyl bromide (**6**) in DMF/ethanol mixture under reflux (Scheme 14) [32].

Shaaban and Elwahy [33] reported the synthesis of bis(5,6-dihydro-*s*-triazolo[3,4-*b*]thiadiazines) (**36**) in good-to-excellent yields by the reaction of bis(*α*-bromoketones) (**28**) with 4-amino-3-mercapto-1,2,4-triazole derivatives (**1**) in ethanol under refluxing conditions (Scheme 15), which, upon reduction with sodium borohydride in refluxing methanol, afforded bis 4-(6,7-dihydro-3-substituted-5*H*-1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine derivatives (**37**) in good yields.

Sayed et al. [34] have reported the synthesis of bis(5,6-dihydro-*s*-triazolo[3,4-*b*] thiadiazines) (**38**) in moderate yields (60–66%) via reaction of bis(*α*-bromoketones) (**28**) with 4-amino-3-mercapto-1,2,4-triazole derivatives (**1**) in catalytic amount of triethylamine in refluxing ethanol-DMF mixture (Scheme 16).

Recently, the synthesis of mono- and bis(triazolothiadiazine) derivatives coupled with benzofuran or benzo[*d*]thiazole moieties (**39**) and (**40**) in good yields (69–79%) has been achieved by the reaction of bromoacetylbenzothiazole/bromoacetylbenzofuran (**6**) with each of 4-amino-3-mercapto-1,2,4-triazoles (**1**) and bis([1,2,4] triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)alkanes (**33**) in catalytic amount of triethylamine in refluxing ethanol (Scheme 17) [35].

In view of the significant biological potential of fused naphthoquinones [36], Khalafy et al. [37] reported the synthesis of a new series of 10-substituted-5*H*-naphtho[1,2-*e*][1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-5-ones (**43**) from the reaction of 4-amino-5-aryl/alkyl-3-mercapto-1,2,4-triazoles (**1**) with 2-bromo-1,4-naphthoquinone (**41**) in ethanol (Scheme 18). This two-step procedure involves the intermediacy of 2-[(4-amino-5-aryl-4*H*-1,2,4-triazol-3-yl)thio]naphthalene-1,4-diones (**42**), which underwent intramolecular cyclization in EtOH-HCl under reflux to afford products (**43**) in 56–82% yields.

2.2 Multicomponent Reaction Approach

Recently, multicomponent reactions (MCRs) due to their high efficiency, atom economy, and reduced time are being explored to access functionalized triazolothiadiazines in a one-pot one-step fashion. In general, construction of triazolothiadiazine scaffold using MCR involves condensation of two components, namely, *α*-halocarbonyl compounds and 4-amino-5-hydrazinyl-3-mercapto-1,2,4-triazole and the third component is being employed for preparing derivatives or variations at hydrazinyl group.

Pavurala and Vedula [38] reported a one-pot multicomponent approach for synthesis of 3-(pyrazolyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl)-2*H*-chromen-2-ones

(**46**) in excellent yields. Reaction of 4-amino-5-hydrazinyl-3-mercapto-1,2,4-triazole (**44**), substituted 3-(2-bromo-acetyl)coumarins (**6**) and acetyl acetone (**45**) in absolute ethanol under reflux afforded (**46**) (Scheme 19). The method has the merits of operational simplicity, good yields and accomplished the synthesis of two heterocycles in one stride.

Keeping in view the importance of pyrazole-linked heterocyclic compounds, Sujata et al. [39] reported one-pot multicomponent synthesis of 3-aryl-1-(6-aryl-7*H*-[1,2,4] triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)-1*H*-pyrazol-5-amines (**48**) and 3-(3-(5-amino-3-aryl-1*H*-pyrazol-1-yl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl)-8-ethoxy-2*H*-chromen-2-ones (**49**). Acetic acid catalyzed condensation of (**44**), various phenacyl bromides (**6**) or 3-(2-bromoacetyl)coumarins (**6′**) and benzoylacetonitriles (**47**) in refluxing ethanol yielded **48** and **49** in excellent yields (Scheme 20).

Aychiluhim et al. [40] described one-pot multicomponent reaction of 3-(2-bromo acetyl)coumarins (**6**), (**44**) and ethyl 2-(2-(aryl)hydrazono)-3-oxobutanoates (**50**) to produce novel 4-(arylhydrazono)-3-methyl-1-(6-(coumarin-3-yl)-7*H*-[1,2,4] triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)-1*H*-pyrazol-5(4*H*)-ones (**51**) (Scheme 21). Short reaction period, higher yields, mild conditions and one-pot synthesis are some of the key features of this methodology.

Keeping the importance of triazolo[3,4-*b*][1,3,4]thiadiazines and phthalazine-1,4-dione, Jilloju et al. [41] explored multicomponent synthesis of 2-(6-phenyl-7*H*-[1,2,4] triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)-2,3-dihydrophthalazine-1,4-dione derivatives (**53**). Three-component reactions of (**44**) with phenacyl bromides (**6**) and phthalic anhydride (**52**) in acetic acid yielded (**53**) in good to excellent yields (Scheme 22).

A simple, multicomponent efficient synthesis of [1,2,4]triazolo[3,4-*b*]thiadiazines (**54**) was reported by Sujatha et al. [42] involving pseudo four component condensation of (**44**), aromatic aldehydes (**2**), and various phenacyl bromides (**6**) in presence of triethyl amine in absolute ethanol to afford novel [1,2,4]triazolo[3,4-*b*]thiadiazine derivatives in excellent yields (Scheme 23). Shorter reaction time, good yields, operational simplicity, mild and clean reaction profiles are some key features of this method.

Thirupaiah et al. [43] have described the synthesis of a pyran and phthalazine substituted [1,2,4]triazolo[3,4-*b*][1,3,4]-thiadiazine derivative (**55**) by multicomponent reaction of equimolar 3-(2-bromoacetyl)-4-hydroxy-6-methyl-2*H*-pyran-2-one (**6**), (**44**), and phthalic anhydrides (**52**) in acetic acid under refluxing conditions (Scheme 24). Similarly, the three-component reaction of **44**, aldehydes (**2**) and 3-(2-bromoacetyl)-4-hydroxy-6-methyl-2*H*-pyran-2-one (**6**), with few drops of acetic acid in absolute ethanol afforded substituted 3-[3-(N[1]-benzylidene-hydrazino)-7*H*-[1,2,4]triazolo[3,4-*b*] [1,3,4]thiadiazin-6-yl]-4-hydroxy-6-methyl-pyran-2-ones (**56**) in good yields.

2.3 Synthesis from Hydrazonoyl Chlorides

Products of the reactions between 4-amino-3-mercaptotriazoles and hydrazonoyl halides are [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines bearing 2-arylhydrazono group at the 7-position. Abdelrazek et al. [44] reported the facile triethylamine-catalyzed synthesis of 3-ethyl-6-methyl-7-(2-arylhydrazono)-7*H*-[1,2,4] triazolo[3,4-*b*][1,3,4]thiadiazine derivatives (**59**) from the reaction of

4-amino-5-ethyl-3-mercapto-1,2,4-triazole (**1**) with variously substituted hydrazo-noyl chlorides (**57**) in refluxing dioxane. *S*-alkylated intermediates (**58**), generated in situ, are cyclized to afford the desired products (**59**) in good yields (80–88%) (Scheme 25).

4-Amino-3-(4-pyridyl)-5-mercapto-1,2,4-triazole (**1**) couples with substituted aryl hydrazonoyl chlorides (**57**) in a similar manner to yield 7-(2-arylhydrazono) [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine derivatives (**60**) (Scheme 26) [45].

2.4 Miscellaneous Reactions

Ebrahimpour et al. [46] synthesized pyrimido[4,5-*e*][1,2,4]triazolo[3,4-*b*][1,3,4]thi-adiazine derivatives (**64**) in good yields via S/N type Smiles rearrangement. Initially, the reaction of 5-bromo-2,6-dichloropyrimidine (**61**) with 4-amino-5-methyl-3-mer-capto-1,2,4-triazole **1** in CH_3CN afforded intermediate 3-((5-bromo-6-chloropyrimi-din-4-yl)thio)-5-methyl-4*H*-1,2,4-triazol-4-amine (**62**) and subsequently nucleo-philic substitution with secondary amines gave corresponding heteroaryl thioethers (**63**). Treatment of (**63**) in refluxing CH_3CN in the presence of $NaNH_2$ afforded the regioisomer **64** instead of expected regioisomer **65** by an intramolecular S_NAr type reaction (Scheme 27). X-ray structural analysis of the product having $NR_2 =$ mor-pholine group demonstrated the formation of **64** through Smiles rearrangement.

A series of pyrimido[5,4-*e*][1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine derivatives (**69**) was synthesized by Afrough et al. [47] through the condensation reaction of 5-bromo-4-[1-(5-bromo-2,6-dichloropyrimidin-4-yl)ethyl]-2-chloro-6-methylpy-rimidine (**66**) with 4-amino-5-methyl-3-mercapto-1,2,4-triazole (**1**) (Scheme 28). This three-step reaction involves the formation of intermediate (**67**), which under-went nucleophilic substitution with secondary amine to give corresponding dihet-eroaryl sulfide intermediates (**68**). Intramolecular S_NAr reaction of compounds **68** in the presence of $NaNH_2$ afforded the desired products (**69**). Interestingly, nucle-ophilic substitution with piperidine provided the desired products in good yields (73–75%) whereas substitution with piperazines resulted in lower yields of products (62–68%).

Skryl'nikova et al. [48] reported the synthesis of 7-benzylidene-3-methyl-6,7-di-hydro-5*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine (**74**) by intramolecular cycliza-tion of 5-methyl-4-(3-phenylprop-2-yn-1-yl)amino-4*H*-1,2,4-triazole-3-thiol (**72**) in DMF at 100 °C in the presence of catalytic amounts of a 40% aqueous solution of KOH (Scheme 29). Compound **72**, in turn, was formed by *p*-toluenesulfonic acid (*p*-TsOH)-catalyzed reaction of 4-amino-3-mercapto-1,2,4-triazole (**1**) with phe-nylpropiolaldehyde (**70**), involving synthesis of 5-methyl-4-[(3-phenylprop-2-yn-1-ylidene)amino]-4*H*-1,2,4-triazole-3-thiol intermediate (**71**), followed by hydro-genation with $NaBH_4$. The hydrogenation with $NaBH_4$ excluded the possibility of formation of side product, 3-methyl-8-phenyl-5,6-dihydro[1,2,4]triazolo[3,4-*b*] [1,3,4]thiadiazepine (**73**).

An efficient synthesis of 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines (**76**) in excellent yields (> 90%) has been presented via a one-pot metal-free protocol by nucleophilic addition of 4-amino-3-mercapto-1,2,4-triazole derivatives (**1**) to dibenzoylacetylene

(DBA) (**75**) under stirring in acetonitrile at room temperature (Scheme 30) by Shah et al. [49]. The structure of product **76** having R = Ph was unambiguously confirmed by X-ray crystallographic data.

Pandey et al. [50] have synthesized a series of novel 5-[(3-aralkyl amido/imidoalkyl) phenyl]-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazine derivatives (**78**) in good yields by condensation reaction of 4-amino-5-mercapto-3-[(3-aralkyl amido/imidoalkyl)phenyl]-1,2,4-triazoles (**1**) with benzoin (**77**) in polyphosphoric acid under reflux (Scheme 31).

The reaction of 4-amino-3-mercapto-5-phenyl-*s*-triazole (**1**) with aromatic/aliphatic/cyclic ketones **79** and **81** in refluxing acetic acid afforded the corresponding triazolo[3,4-*b*][1,3,4]thiadiazines **80** and **82** in a single step (Scheme 32) [51]. This method is better than classical methods in terms of synthesis of 6- and 7-substituted derivatives (rare), and avoidance of use of highly toxic and irritating halo compounds.

A base catalyzed condensation reaction of 4-amino-3-[(4-nitrobenzyl) thio]-4*H*-1,2,4-triazoles (**1**) with alkylisatins (**83**) in ethanol was described by Gaponenko et al. [52]. Spirocyclic triazolo[3,4-*b*][1,3,4]thiadiazine derivatives (**85**) were obtained by the formation of new C–C bond in the final step of diastereospecific heterocyclization of intermediate **84** (Scheme 33).

Prakash et al. [53] synthesized a series of 3-alkyl/aryl-7/9-methyl-10,10*a*-dihydroindeno[1,2-*e*][1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines (**87**) (67–93% yields) by the cycloconsensation of 4/6-methyl-2-tosyloxy-1-indanones (**86**), generated by oxidation of 4/6-methyl-1-indanones with [(hydroxy)tosyloxyiodo]benzene (HTIB) in acetonitrile, and 3-alkyl/aryl-4-amino-5-mercapto-1,2,4-s-triazoles (**1**) in refluxing ethanol (Scheme 34).

3 Pharmacological Properties of 1,2,4-Triazolo[3,4-*b*][1,3,4] thiadiazines

Reported literature protocols have provoked researchers to discover new biological active compounds consisting of triazolothiadiazine as a core motif. 1,2,4-Triazolo[3,4-*b*][1,3,4]thiadiazine derivatives have been reported to possess significant pharmacological activity as anticancer, antimicrobial, analgesic and anti-inflammatory, antioxidant, antiviral, enzyme inhibitors and antitubercular agents through different mechanisms of action, such as enzyme inhibition, interaction with intracellular targets, etc. Triazolothiadiazines also find applications in other fields, such as intermediates for photographical couplers [54, 55], PDE4 inhibitors [56], and corrosion inhibitors [57, 58].

3.1 Anticancer Agents

Aytaç et al. [59] designed and synthesized a series of 1,2,4-triazolo[3,4-*b*][1,3,4] thiadiazines (**88**) linked with structural motifs of nonsteroidal analgesic anti-inflammatory drugs (ibuprofen, naproxen and flurbiprofen) at the 3-position. All

synthesized derivatives were screened for their in vitro cytotoxic potential against liver (Huh7), breast (MCF-7) and colon (HCT116) cancer cell lines. Preliminary results indicated that all triazolothiadiazine derivatives exhibited significant cytotoxic potential ($IC_{50} = 1.1–18.8$ μM) than synthon, 4-amino-1,2,4-triazole-5-thiones ($IC_{50} = 20.2–67.9$ μM), thereby implying that fusion of triazole with thiadiazine is crucial for cytotoxic potential. Among triazolothiadiazines, compounds **88a–j** with lower IC_{50} values were further evaluated against hepatocellular carcinoma cell lines (HepG2, Hep-3B, Mahlavu, FOCUS and Snu475) and were found to display substantial cytotoxic activities ($IC_{50} = 1.5–10.1$ μM) (Fig. 3). According to fluorescent microscopy and flow cytometry analysis, the compounds **88a–c**, **88f** and **88j** were found to induce apoptosis and SubG1 arrest in Huh7 cells. Mechanistic study revealed that compounds **88b** and **88c** initiated oxidative stress induced apoptosis through ASK-1 protein activation and Akt protein inhibition.

Ahmad et al. [60] synthesized a series of 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines (**89**) having an alkenyl/hydroxyalkenyl fatty acid chain and evaluated them for their in vitro anticancer activity against three different human cancer cell lines, namely Hep3 B, MCF-7, HeLa (human cervical carcinoma) and toxicity against PBMCs (peripheral blood mononuclear cells). All the synthesized compounds were found to be non-toxic against PBMCs. SAR studies revealed that compounds **89c** and **89d** containing a hydroxyl group on a fatty acid chain exhibited maximum cytotoxic potential ($IC_{50} = 6.0–8.8$ μM) against all the three tested cancer cells, as depicted in Fig. 4.

Xu et al. [61] synthesized novel 3,6-diaryl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4] thiadiazine derivatives (**90**) as anti-proliferative agents against three human cancer cell lines: gastric adenocarcinoma (SGC-7901), lung adenocarcinoma (A549) and fibrosarcoma (HT-1080) cells. From the series, compounds **90a–h** exhibited excellent potency against SGC-7901, A549 and HT-1080 cell lines with IC_{50} values at the nanomolar level ($IC_{50} = 0.011–0.079$ μM). A SAR study revealed
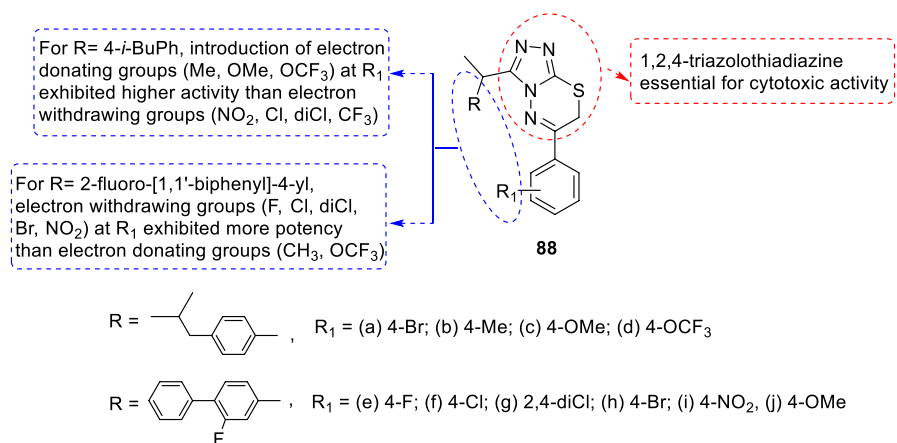
Fig. 3 Structure–activity relationship (SAR) studies of non-steroidal drugs linked to 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines as anticancer agents
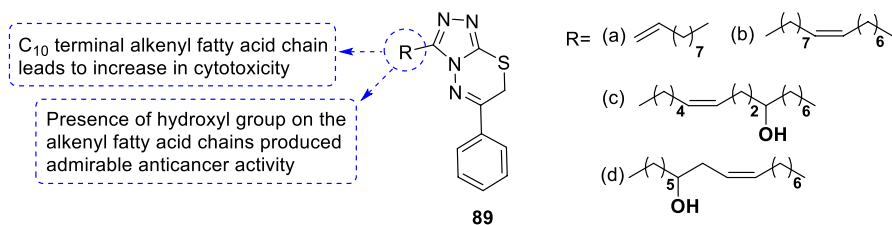
Fig. 4 SAR analysis of fatty-acid-chain-substituted 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines as anticancer agents

the significance of substitution at position-3 of the triazolothiadiazine scaffold as shown in Fig. 5. Compound **90g** displayed the most promising antiproliferative activity against the three human cancer cell lines, with an IC_{50} value of 0.011–0.015 µM and effectively induced cell cycle arrest at G2/M phase in SGC-7901 cells. This compound effectively inhibited tubulin polymerization, with an IC_{50} value of 1.6 µM. Docking studies revealed that compound **90g** binds to the tubulin perfectly at the colchicine binding site.

Khan et al. [62] synthesized a series of 6-aryl-3-(pyridin-4-yl)-7*H*-[1,2,4] triazolo[3,4-*b*][1,3,4]thiadiazines (**91**) and screened their anticancer activity against kidney fibroblast (BHK-21) and lung carcinoma (H157) cell lines at four different concentrations (100, 10, 1 and 0.1 µM) by cell growth inhibition. Among the tested series, compound **91c** showed the highest potency, with 78.6% inhibition (100 µM) against H157 cells, which was better than the standard drug vincristine (74.5%). Compounds **91a** and **91d** exhibited excellent cytotoxicity against BHK-21 cells with 73.3% and 72.6% inhibition (100 µM). SAR studies presented the significance of aryl substituents at position-6 of triazolothiadiazine for anticancer activity and substitution of C6 by biphenyl group significantly decreased anticancer activity (Fig. 6).

In another work, Khan et al. [63] synthesized 6-aryl-3-(pyridin-3-yl)-7*H*-[1,2,4] triazolo[3,4-*b*][1,3,4]thiadiazine derivatives (**92**) and screened their cytotoxic potential against lung carcinoma (H157) and vero cell lines. Compound **92 h** (R = pyridin-3-yl, Ar = 3,4-diClPh) presented excellent antiproliferative activity against H157 cell lines with an IC_{50} value of 0.96 ± 0.43 µM at 1 µM concentration as compared with the standard drug vincristine (IC_{50} = 1.03 ± 0.04 µM) (Fig. 6).
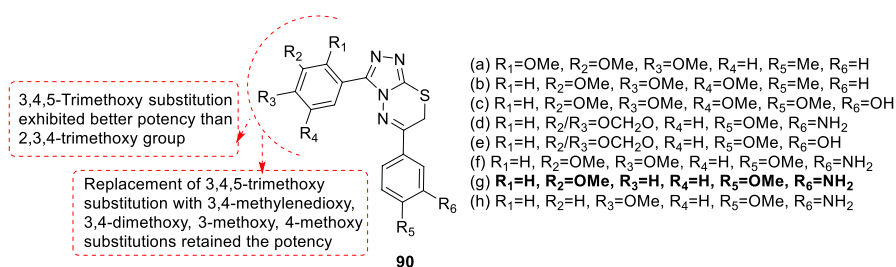
Fig. 5 SAR exploration of 3,6-diaryl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines as anticancer agents
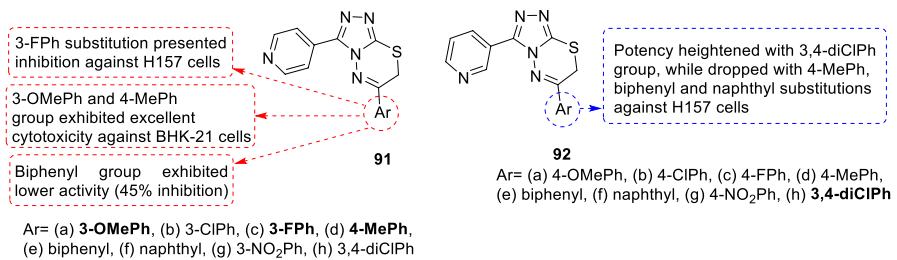
Fig. 6 SAR analysis and anticancer activity of 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines against BHK-21 and H157 cell lines

Kamel and Abdo [64] accomplished the synthesis of pyridine linked 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines (**93**) and investigated in vitro anticancer activity against six human cancer cell lines, namely human gastric cancer (NUGC), human colon cancer (DLD1), human liver cancer (HA22T and HEPG2), nasopharyngeal carcinoma (HONE1), human breast cancer (MCF) and normal fibroblast cells (WI38). Among them, compound **93a** displayed significant cytotoxic potential ($IC_{50} = 59$–469 nM) against all the tested cancer cell lines except the cell line DLD1($IC_{50} = 2350$ nM) (Fig. 7).

Zhang et al. [65] synthesized novel 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines (**94**) bearing furan and thiophene nucleus and evaluated them for their antiproliferative activities against four human cancer cell lines, namely PC-3 (prostate cancer cells), HepG2 (hepatoma cells), A549 (alveolar epithelial cells), and MCF-7 (mammary adenocarcinoma cells). Results indicated that most of the tested compounds showed good-to-excellent antiproliferative activities against PC-3 and A549 cell lines. A SAR study revealed that thiophene derivatives displayed higher cytotoxic potential against four tested cell lines than furan derivatives (Fig. 8). Compound **94b** exhibited 2- to 11-fold higher activity against PC-3, HepG2 and A549 cells ($IC_{50} = 5.09$–12.74 μM) than the standard drug fluorouracil ($IC_{50} = 22.28$–46.83 μM) and arrested G2/M cell-cycle in PC-3 cells in a dose-dependent manner.

Farghaly et al. [66] carried out the synthesis and anticancer evaluation of 3,6,7-trisubstituted-1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines (**95**). Among them, compound **95a** exhibited potential activity against both HEPG-2 and HCT cell lines, with IC_{50} values of 8.63 and 8.33 μg ml^{-1}, respectively, whereas compound **95h** presented excellent activity against HEPG-2 cell line with an IC_{50} value of 5.63 μg ml^{-1} (Fig. 9).
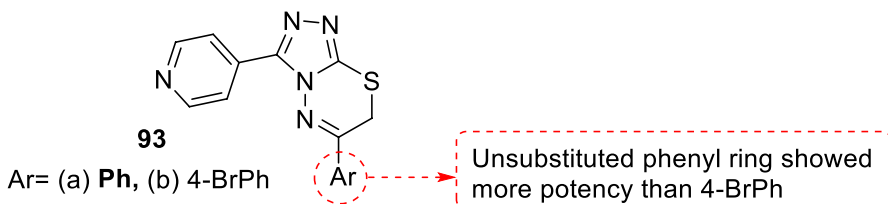
Fig. 7 Pyridine linked 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines as anticancer agents
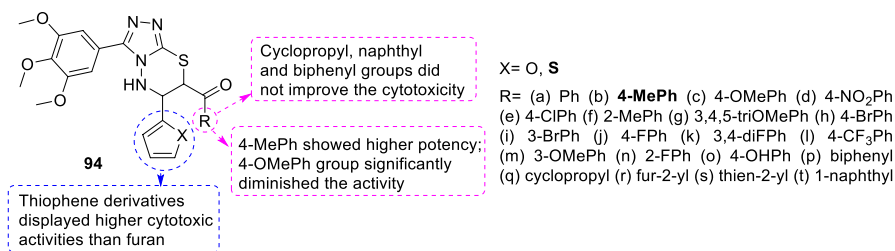
Fig. 8 SAR of thiophene/furan linked 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines as anticancer agents

Through an HTS process, LaPorte et al. [67] identified a series of pyrazole-linked 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines (**96**) as potent and selective STAT3 inhibitors. STAT3 is an attractive target for anticancer agents. Among the tested series, compounds **96a** and **96b** displayed significant antiproliferative activity against several head and neck squamous cell carcinoma (HNSCC) cell lines, namely 686LN, Cal33, FaDu, and OSC19 ($GI_{50} = 14$–44 μM) by reduction of phosphorylated STAT3 levels and downstream effects on STAT3 gene expression targets. Compounds **96a** and **96b** proved to be selective inhibitors STAT3 with IC_{50} values of 6.8 and 9.6 μM, respectively, over STAT1 (IC_{50}: > 50 μM for each). SAR studies revealed the significance of pyrazole group and specific aryl substitution on the thiadiazine for inhibition of STAT3 pathway as shown in Fig. 10. Further, improvements in potency and metabolic stability were accomplished by the introduction of α-methyl group on the thiadiazine (**96c**, $GI_{50} = 1.9$ μM, $IC_{50} = 8.2$ μM).

Sever et al. [68] synthesized a series of indomethacin based 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine derivatives (**97**) and evaluated their cytotoxic potential against the human glioma cell line T98. Among the evaluated series, compound **97h** (Fig. 11) exhibited the most potent anti-tumor activity via inhibition of COX-2 mRNA levels in dose-dependent manner. The results of flow cytometric analysis demonstrated that apoptosis stimulating percentages of compound **97h** at 50 μM and 100 μM doses were calculated as 11% and 12%, respectively, in comparison with the control group (1.1%). Docking analysis revealed compound **97h** and indomethacin were found to bind similarly to the active site of the COX-2 enzyme.

Poojary et al. [69] synthesized a series of 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines (**98**) possessing arylfurfuryl, 2,4-dichloro-5-fluorophenyl and aryloxymethyl/anilinomethyl moieties (**98a–h**) and screened compounds **98d** and **98h** (Fig. 12) for their anticancer potential initially against NCI-H460 (lung), MCF-7 (breast) and SF
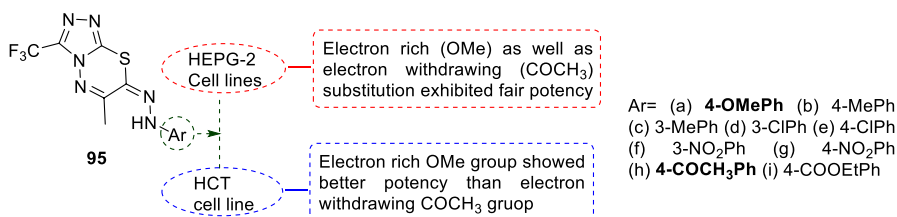
Fig. 9 SAR evaluation of 3,6,7-trisubstituted-1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines as anticancer agents
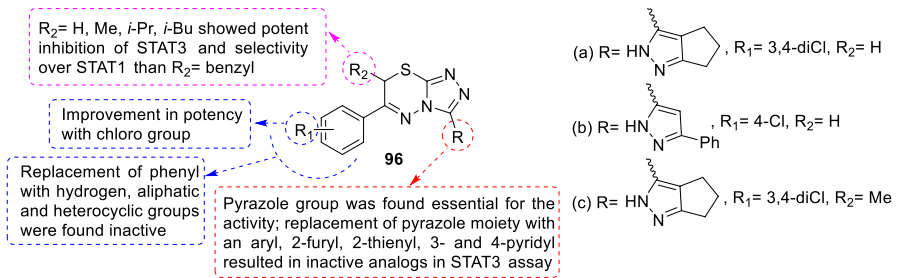
Fig. 10 SAR analysis and anti-proliferative activities of pyrazole-containing 1,2,4-triazolo[3,4-*b*][1,3,4] thiadiazines as STAT3 inhibitors
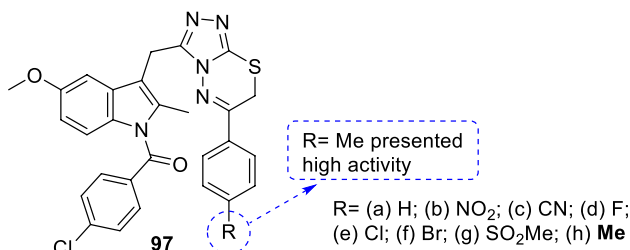
Fig. 11 Anti-tumor activity of indomethacin based 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines
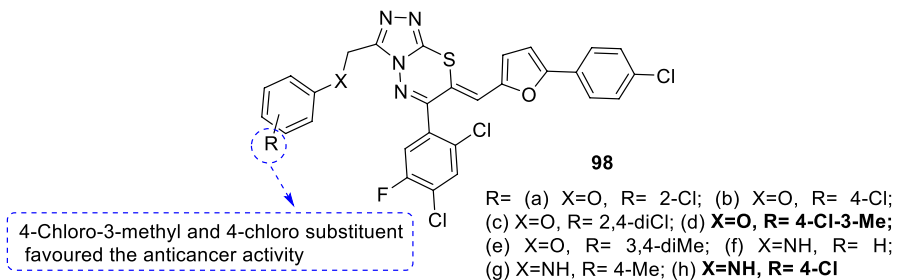
Fig. 12 Anticancer activities of 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines

268 (CNS) cell lines. Further evaluation of compounds **98d** and **98h** against a full panel of 60 cell lines indicated that compound **98d** showed good activity against SW-620 (colon cancer), MALME-3 M (melanoma) and T-47D (breast cancer) cell lines with GI_{50} values of 3.1, 3.8 and 7.9 μM, respectively, whereas compound **98h** showed good activity against MMOLT-4 (leukemia), EKVX (non-small cell lung cancer) and NCI/ADR-RES (breast cancer) cell lines with GI_{50} values of 3.3, 7.5 and 6.4 μM, respectively.

A series 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines (**99**) was prepared by Fan et al. [70] and evaluated for their in vitro anticancer activity against two prostate cancer cell lines PC-3 and DU-145 using SRB (sulforhodamine B) and MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay. All the
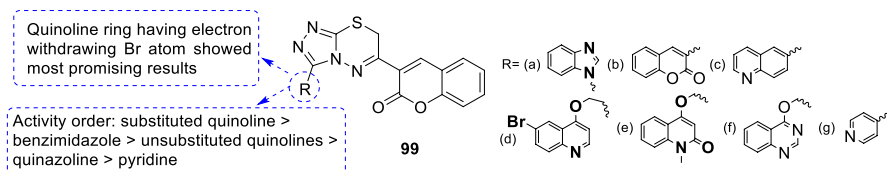
Fig. 13 SAR examination of 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines with different heteroaromatics as anticancer agents
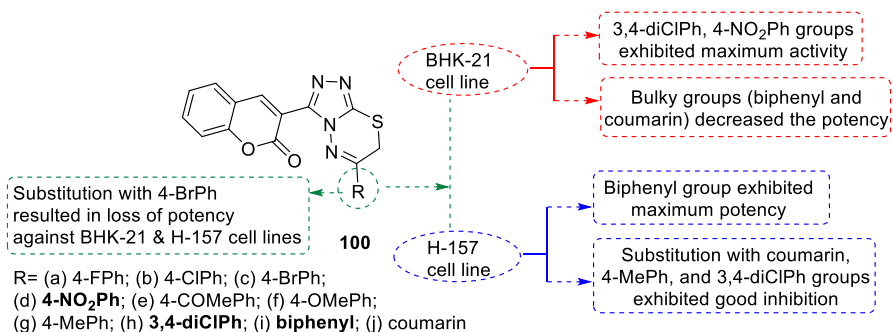
Fig. 14 SAR studies and anticancer evaluation of coumarin-linked 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines

synthesized compounds exhibited remarkable cytotoxicity against targeted cell lines with GI_{50} values ranging from 12.0 to 32.5 μg ml^{-1} and 16.5–27.7 μg ml^{-1} against DU-145 and PC-3 cell lines, respectively. SAR studies revealed that compounds **99a**, **99d** and **99e** combined with benzimidazole and quinoline moieties exhibited the most promising activity, with 63, 71 and 66% cytotoxicity, respectively (Fig. 13).

Ibrar et al. [71] designed several coumarin-triazolothiadiazine hybrids (**100**) and screened their in vitro anticancer activity against kidney fibroblast (BHK-21) and lung carcinoma (H-157) cell lines. Among the series, compounds **100d** and **100h** showed the highest inhibition against BHK-21 cell lines ($IC_{50=}$1.24 and 1.39 μM), while compound **100i** showed the highest rate of inhibition against the H-157 cell line (IC_{50}1.01 μM), greater than the standard drugs vincristine ($IC_{50} = 1.03$ μM) and cisplatin ($IC_{50} = 1.31$ μM). A SAR study revealed the significance of substituents as shown in Fig. 14.

Yakaiah et al. [72] carried out synthesis of 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines (**101**) containing coumarin and benzotriazole moieties and evaluated their anticancer against a human lung cancer (HCC 827) cell line. Bioassay results indicated that compound **101k** exhibited promising activity, with an IC_{50} value of 0.821 μM. SAR studies indicated that the presence of an electron-rich group on the coumarin ring was responsible for better activity, as shown in Fig. 15.

Iqbal et al. [73] reported the synthesis of coumarin-triazolothiadiazine conjugates (**102**) and explored them for their cytotoxic potential against three different cancer cell lines: breast cancer cells (MCF-7), bone-marrow cancer cells (K-562), and

(a) R=R$_1$=R$_2$=H; (b) R= *t*-butyl, R$_1$=H, R$_2$=*t*-butyl;
(c) R=H, R$_1$=H, R$_2$=Cl; (d) R=H, R$_1$=H, R$_2$=Br;
(e) R=OMe, R$_1$=H, R$_2$=H; (f) R=OMe, R$_1$=H, R$_2$=Br;
(g) R=H, R$_1$=H, R$_2$=N(Et)$_2$; (h) R=NO$_2$, R$_1$=H, R$_2$=NO$_2$;
(i) R=Br, R$_1$=H, R$_2$=Br; (j) R=H, R$_1$=OH, R$_2$=H;
(k) **R/R$_1$/R$_2$= 5,6-benzo**

Presence of electron rich groups (5,6-benzo, OMe, OH) on coumarin ring was necessary for cytotoxic activity
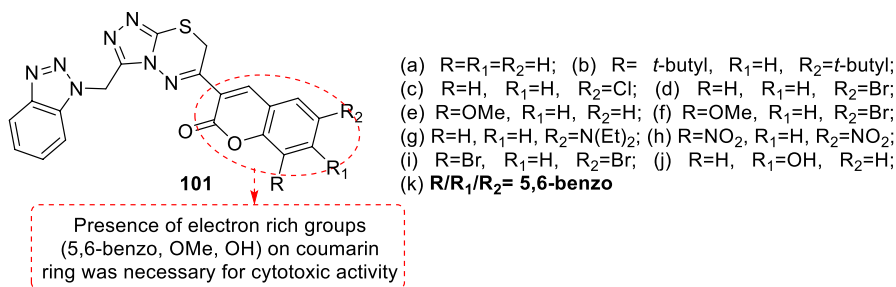
Fig. 15 Benzotriazole and coumarin-combined triazolothiadiazine as anticancer agent

Substitution with 4-FPh group showed maximum activity

Introduction of 4-MePh group showed best potency against HeLa cell line (IC$_{50}$= 12.9 µM)
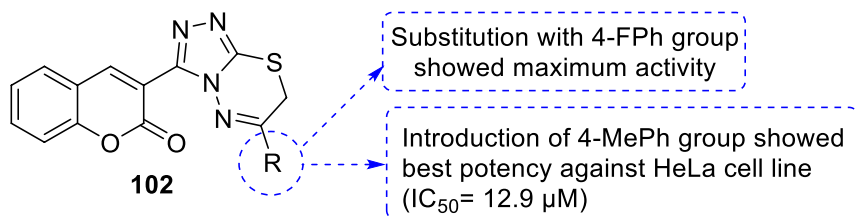
R= (a) **4-FPh**; (b) 4-ClPh; (c) 4-BrPh; (d) 4-NO$_2$Ph; (e) 4-COMePh;
(f) 4-OMePh; (g) **4-MePh**; (h) 3,4-diClPh; (i) biphenyl; (j) coumarin

Fig. 16 Coumarin-triazolothiadiazines with anticancer activity

cervical cancer cells (HeLa). Among the tested series, compounds **102a** and **102g** exhibited a significant anti-proliferative effect in cancer cells (IC$_{50}$ = 2.21–21.2 µM). Moreover, compound **102a** with an IC$_{50}$ value of 2.21 µM was found to be more potent than the standard drug carboplatin (IC$_{50}$ = 3.91 µM) against MCF-7 cell lines, and this compound induced 50.1% apoptosis in via G$_2$/M phase arrest in MCF-7 cells. A SAR study was carried out to correlate the importance of substituents with the observed activity (Fig. 16).

Arandkar and Vedula [74] introduced a series of 1,2,4-triazolo[3,4-*b*][1,3,4]thia-diazine derivatives (**103**) and screened them for anticancer activity at a concentration 10^{-5} M against 60 cancer cell lines comprising leukemia, non-small cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian, renal, prostate cancer and breast cancer lines. A SAR study revealed that compounds containing halo groups exhibited higher potency than other groups (Fig. 17). From the tested compounds **103d**, **103e**, **103f** and **103l** exhibited significant activity against renal cancer OU-31 cell line with 47.42%, 54.64%, 46.76 and 48.14% growth, respectively. Compound **103f** also exhibited significant activity on leukemia MOLT-4 cell line with 49.82% growth.

Mohamady et al. [75] designed a series of naphthoquinone-hydrazino[1,2,4] triazolo[3,4-*b*]-1,3,4-thiadiazine derivatives (**104**) and screened their cytotoxicity against breast cancer cell lines (MCF-7) and hepatocellular carcinoma cell lines (HepG2) via MTT assay. Among the tested compounds, compound **104a** (Fig. 18)

(a) $R_1=R_2=R_3=R_4=H$
(b) $R_1=H$; $R_2=Me$; $R_3=H$; $R_4=Me$
(c) $R_1=H$; $R_2=OMe$; $R_3=H$; $R_4=OMe$
(d) $R_1=H$; $R_2=Cl$; $R_3=H$; $R_4=Cl$
(e) $R_1=H$; $R_2=Br$; $R_3=H$; $R_4=Br$

(f) $R_1=H$; $R_2=F$; $R_3=H$; $R_4=F$
(g) $R_1=Cl$; $R_2=Cl$; $R_3=Cl$; $R_4=Cl$
(h) $R_1=H$; $R_2=Ph$; $R_3=H$; $R_4=Ph$
(i) $R_1=H$; $R_2=NO_2$; $R_3=H$; $R_4=NO_2$
(k) $R_1=H$; $R_2=H$; $R_3=H$; $R_4=F$
(l) $R_1=H$; $R_2=Cl$; $R_3=H$; $R_4=F$

Presence of halogen substituents significantly increased the activity

103
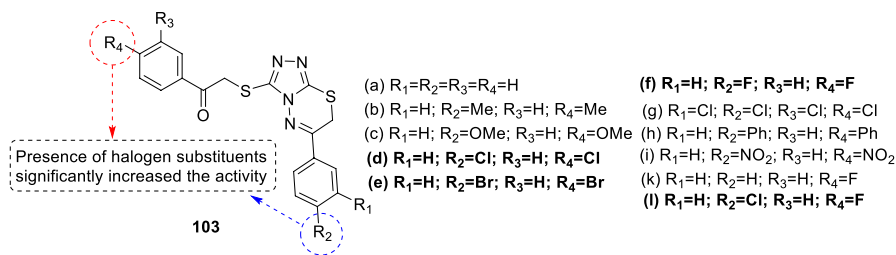
Fig. 17 SAR inspection and anticancer activities of triazolothiadiazine analogs

exhibited maximum cytotoxicity against HepG2 cell lines ($IC_{50}=0.07$ μM) and significantly upregulated caspase 3, with induction of apoptosis at pre-G1 phase and cell death at G2/M phase. Compounds **104a–c** exhibited significant inhibition of Topoisomerase-IIβ (0.55–0.64 μM). Docking analysis explained the potential interactions of **104a–c** within the ATP catalytic binding domain of Topo-IIβ.

Recently, Çevik et al. [76] synthesized a series of benzimidazole clubbed 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine derivatives (**105**) and tested them for their in vitro anticancer activity against MCF-7 cancer cell lines, among which compounds **105c**, **105e**, and **105k–l** exhibited significant anticancer activity ($IC_{50}=0.016–0.119$ μM). Moreover, compounds **105e** and **105l** were found to be more active ($IC_{50}=0.016$ and 0.018 μM, respectively) than the standard drug cisplatin ($IC_{50}=0.020$ μM). A SAR study explained the effect of substituents (Fig. 19).

Puthiyapurayil et al. [77] synthesized several 6-arylsubstituted-3-[2-(4-substitutedphenyl)propan-2-yl]-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines (**106**) and evaluated them for their in vitro cytotoxicity against MCF-7 and A549. Among the screened series, compound **106h** and **106i** showed most promising cytotoxicity, with an IC_{50} value 10.54 and 14.28 μM, respectively, against the MCF-7 cell line. A SAR study showed the significance of substituents for favorable activity (Fig. 20).

Several 6-ferrocenyl-3-subsituted-7*H*-1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines (**107**) were synthesized and evaluated biologically against human fibrosarcoma cells

Activity order against HepG2:
$Cl>Br>OMe>Ph>CF_3>H>Me$

Activity order against MCF-7:
$Ph>Me>Br>Cl>H>OMe$

Unsubstituted phenyl decreased the antitumor activity

104

R= (a) H; (b) OMe; (c) Cl;
(d) Me; (e) Br; (f) Ph; (g) CF_3
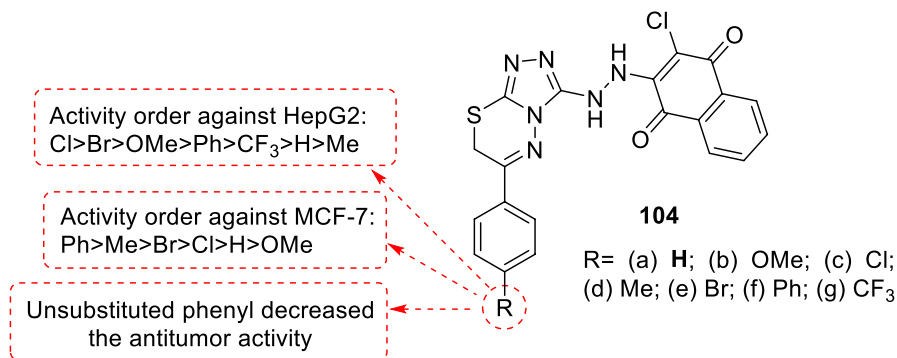
Fig. 18 SAR study of naphthoquinone-hydrazinotriazolothiadiazines as anticancer agents
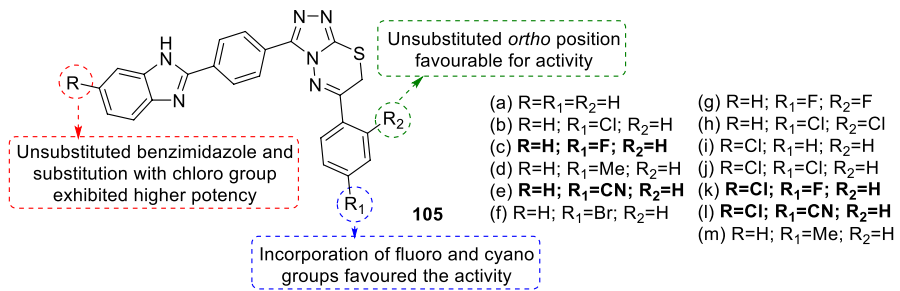
Fig. 19 SAR of naphthoquinone-hydrazinotriazolothiadiazines as anticancer agents

(HT1080) and human foreskin fibroblasts (hTERT-BJ) cell lines by Miao et al. [78]. Compounds **107a** and **107b** (Fig. 21) exhibited a higher inhibitory effect on HT1080 cells than hTERT-BJ cells. Bioassay results revealed that compounds **107a** and **107b** suppressed the viability of HT1080 cells by inducing cell cycle arrest in G1 phase, apoptosis, decreasing mitochondrial membrane potential and increasing the ratio of Bax/Bcl-2.

Ansari et al. [79] synthesized 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine deriva-tives (**108**) bearing the 3,4,5-trimethoxyphenyl moiety and screened their anti-proliferative potential against three human cancer cell lines, namely adenocarci-nomic human alveolar basal epithelial cells (A549), breast cancer cells (MCF7), and human ovarian carcinoma cells (SKOV3). Among them, three compounds **108a** ($IC_{50} = 0.70$ μM), **108m** ($IC_{50} = 0.30$ μM) and **108** ($IC_{50} = 0.60$ μM) were significantly more potent than the reference drug etoposide ($IC_{50} = 1.89–2.99$ μM)
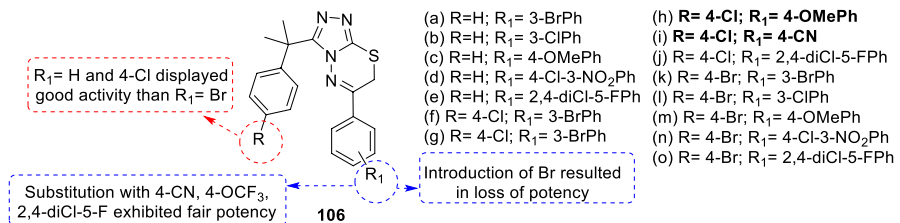
Fig. 20 SAR and anticancer potential of 6-arylsubstituted-3-[2-(4-substitutedphenyl)propan-2-yl]-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines
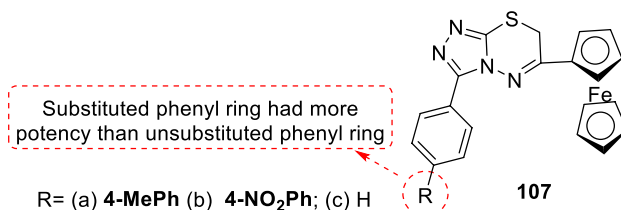
Fig. 21 Anti-tumor activity of 6-ferrocenyl-3-subsituted-7*H*-1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines

(a) **R= H; R$_1$= 3,4,5-triOMe**   (h) R= H; R$_1$= 4-OMe
(b) R= 4-Me; R$_1$= 3,4,5-triOMe   (i) R= 4-Me; R$_1$= 4-OMe
(c) R= 4-OH; R$_1$= 3,4,5-triOMe   (j) R= 3,4,5-triOMe; R$_1$= 4-Ph
(d) R= 3-Br; R$_1$= 3,4,5-triOMe   (k) R= 3,4,5-triOMe; R$_1$= H
(e) R= 4-OMe; R$_1$= 3,4,5-triOMe   (l) R= 3,4,5-triOMe; R$_1$= 3,4,5-triOMe
(f) R= 4-Cl; R$_1$= 3,4,5-triOMe   (m) **R= 3,4,5-triOMe; R$_1$= 4-Br**
(g) R= 2,4-diCl; R$_1$= 3,4,5-triOMe   (n) R= 3,4,5-triOMe; R$_1$= 4-OH
(o) **R= 3,4,5-triOMe; R$_1$= 4-Me**

108

3,4,5-triOMe group at R or R$_1$ exhibited potent activity; replacement of 3,4,5-triOMe group with 4-OMe decreased potency
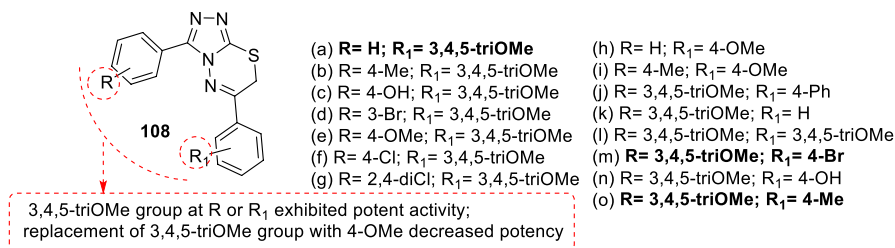
Fig. 22 SAR and anticancer activities of 3,4,5-trimethoxyphenyl linked 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines

Substitution with CH$_2$COPh group resulted more potency

R=H exhibited moderate activity

R= (a) H; (b) **CH$_2$COPh**   **109**
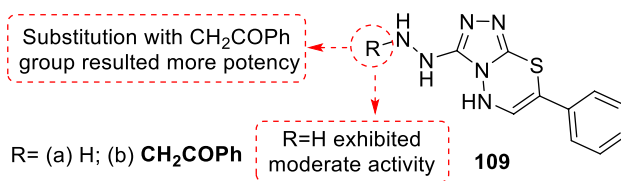
Fig. 23 SAR study of 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine derivatives as potent anticancer agents

against MCF7, SKOV3 and A549 cells, respectively. SAR studies revealed the significance of trimethoxy substitution for anti-tumor activity as shown in Fig. 22. Moreover, compounds **108a** and **108m** exhibited inhibitory potential on tubulin polymerization with IC$_{50}$ values of 1.03 and 1.61 μM, respectively. Docking studies also indicated that compounds **108a**, **108m**, **108o** inhibited tubulin polymerization through binding to the colchicine-binding site.

Radwan et al. [80] evaluated anticancer activity 1,2,4-triazolo[3,4-*b*][1,3,4] thiadiazine derivatives **109a** and **109b** against liver human cancer cell lines (HEPG2) in vitro (Fig. 23). Compounds **109a** and **109b** with IC$_{50}$ values of 31.1 μM and 19.6 μM, respectively, were more effective than reference drug doxorubicin (IC$_{50}$ = 36.3 μM). The most active compound **109b** exhibited in vivo anti-hepatocellular carcinoma efficacy in albino mice via regulating tumor angiogenesis by reducing the level of vascular endothelial growth factor (VEGF) by 30% and restoring the activities of hepatic marker enzymes, hepatic tyrosine kinase (HTK), aspartate and alanine-aminotransferase (AST and ALT) as well as improved the liver levels of lipid peroxides (by 62%) and glutathione (GSH) (by 99%).

Liu et al. [81] synthesized and screened 3-(3-methoxyphenyl)-6-(3-amino-4-methoxyphenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine **110** for its cytotoxic potential against fibroblast (L929), alveolar basal epithelial (A549), cervical carcinoma (HeLa), fibrosarcoma (HT-1080), breast (MCF-7) and gastric (SGC-7901) cell lines. Compound **110** exhibited significant cytotoxicity against HeLa and SGC-7901 cells, with IC$_{50}$ values of 0.11 and 0.12 μM, respectively (Fig. 24). Immunofluorescence and flow cytometry studies revealed that compound **110** induced cell cycle arrest in G2/M phase. Western blot analysis and examination of mitochondrial membrane potential revealed that compound **110** induced apoptosis in SGC-7901 cells

through mitochondria-mediated and a death receptor-mediated pathway, whereas in HeLa cells induction was mainly via a mitochondria-mediated pathway.

Ma et al. [82] synthesized a series of [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine derivatives (**111**) and studied in vitro anticancer activity against four human cancer cell lines: colorectal adenocarcinoma (HT-29), cervical carcinoma (HeLa), lung adenocarcinoma (A549) and breast (MCF-7) cell lines. Among them, compound **111j** was found to have significant cytotoxic (IC$_{50}$ = 0.004–0.098 μM) and anti-tubulin (IC$_{50}$ = 1.69 μM) properties and also exhibited excellent selectivity with no cytotoxicity over the normal human embryonic kidney (HEK-293) cell line (IC$_{50}$ > 100 μM). SAR studies revealed the significance of substitution on the thiadiazine for better potency as shown in Fig. 25. Compound **111j** induced cell cycle arrest in A549 cells at G2/M phase by regulation of p-cdc2 and cyclin B1 expressions, and subsequently apoptosis via up-regulating cleaved PARP, activation of caspase-3 and down-regulating Bcl-2 expression. Molecular modeling studies revealed that compound **111j** significantly blocked tubulin polymerization through binding to the colchicine site of tubulin. Moreover, compound **111j** displayed promising in vivo activity in an A549 lung cancer xenograft mouse model.

Ji et al. [83] introduced spirooxindole derivatives of [1, 2, 4]triazolo[3,4-*b*][1,3,4]thiadiazine (**112**) and evaluated their cytotoxic potential. Among the evaluated series, compound **112d** was found to exhibit 3.0, 1.6, 2.7 and 1.3 times more potency than the standard drug 5-fluorouracil, with IC$_{50}$ values of 8.02, 6.62, 9.49 and 17.65 μM against prostate (DU145), esophageal (EC109), gastric carcinoma (MGC803) and breast (MCF-7) cell lines, respectively. A SAR study revealed the significance of substituents, as shown in Fig. 26.

Ismail et al. [84] synthesized 6-aryl-3-(aryl/hetarylbenzylidene)hydrazineyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines (**113**) and studied their anticancer potential against full NCI-60 cancer cell lines. Compound **113h** was found to show the highest percent inhibition over the 60 cell lines, with mean growth inhibition percentage of 45.44%. SAR studies revealed that compounds with *p*-cholorophenyl substitution at C-6 postion of triazolothiadiazine nucleus had better activity than analogs with an unsubstituted phenyl ring (Fig. 27). Mechanistic

Fig. 24. 3-(3-Methoxyphenyl)-6-(3-amino-4-methoxyphenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine as potent anticancer agent

110

R= (a) PhNH; (b) 4-BrPhNH; (c) 4-FPhNH;
(d) 3-FPhNH; (e) 2-MePhNH; (f) 2,6-diClPhNH;
(g) PhCH$_2$NH; (h) Ph; (i) 4-OMePh; (j) **4-MePh;**
(k) 3-MePh; (l) 2-MePh; (m) Me; (n) Et; (o) biphenyl;
(p) 1-naphthyl; (q) chromen-2-one; (r) fur-2-yl;
(s) thien-2-yl; (t) cyclopropyl

Substituents at *para* position of phenyl ring were found to be more favourable for activity than that of *ortho/meta*-position

Replacement of the phenyl groups with alkyl chains (Me or Et) led to slight decrease of potency

Replacement with naphthalene or heterocycle cores did not lead to a noticeable effect on activity.

Introduction of cyclopropyl led to the dramatical loss of activity

Fig. 25 SAR study of 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine derivatives as potent anticancer agents

Substituents at *para* position of phenyl ring were found to be more favourable for activity than that of *meta* position

Replacement of the phenyl groups with ethyl or hydrogen decreased the potency

Unsubstituted phenyl ring was crucial for the activity; phenyl ring substituted with electron-donating (OMe) or electron-withdrawing group (Cl) lowered the activity

(a) R= Et; R$_1$= OMe, (b) R= Et; R$_1$= H, (c) R= 4-OMePh; R$_1$= H, (d) **R= 4-MePh; R$_1$= H,**
(e) R= 3-ClPh; R$_1$= OMe, (f) R= 3-MePh; R$_1$= H, (g) R= H; R$_1$= H, (h) R= H; R$_1$= OMe,
(i) R= 3-MePh; R$_1$= OMe, (j) R= 3-MePh; R$_1$= Cl, (k) R= 4-OMePh; R$_1$= Cl, (l) R= Ph; R$_1$= OMe

Fig. 26 SAR study of spirooxindoles incorporating 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine derivatives as potent anticancer agents

Replacement with biphenyl or 2-furyl resulted in loss of potency

Potency heightened with 4-CF$_3$Ph

Unsaturation is essential for activity

Presence of chlorine is admirable for activity

Unfused triazole is inactive, fused 1,2,4-triazolothiadiazine essential for activity

(a) R= H; R$_1$= Ph
(b) R= H; R$_1$= 4-CF$_3$Ph
(c) R= H; R$_1$= 3-OMePh
(d) R= H; R$_1$= *N,N*-diMe
(e) R= H; R$_1$= biphenyl
(f) R= H; R$_1$= fur-2-yl

(g) R= 4-Cl; R$_1$= Ph
(h) **R= 4-Cl; R$_1$= 4-CF$_3$Ph**
(i) R= 4-Cl; R$_1$= 3-OMePh
(j) R= 4-Cl; R$_1$= *N,N*-diMe
(k) R= 4-Cl; R$_1$= biphenyl
(l) R= 4-Cl; R$_1$= fur-2-yl

Fig. 27 SAR and anticancer study of 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine derivatives

studies demonstrated that compound **113h** selectively inhibited the glycogen synthase kinase-3β (GSK-3β), with IC$_{50}$ = 0.883 μM and increased active caspase-3 levels. Further, compound **113h** induced cell cycle arrest in G2/M phase and apoptosis in PC-3 prostate cancer-treated cells.

Hu et al. [85] synthesized [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine derivatives (**114**) linked with antibacterial fluoroquinolones (FQ) at the 3-position. All synthesized derivatives were screened for their in vitro cytotoxic potential against murine leukemia (L1210) and Chinese hamster ovary (CHO) cell lines. Compound **114b**

Fig. 28 SAR study of 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine derivatives as potent anticancer agents

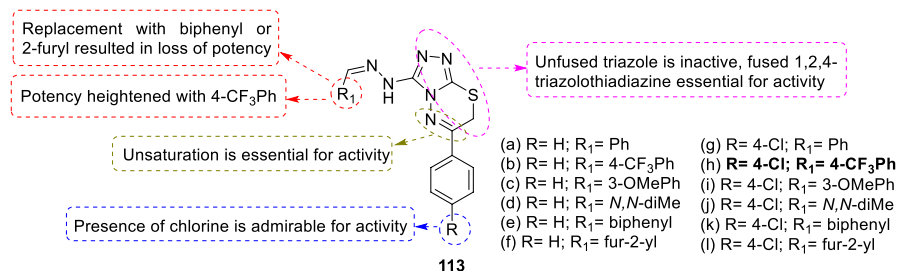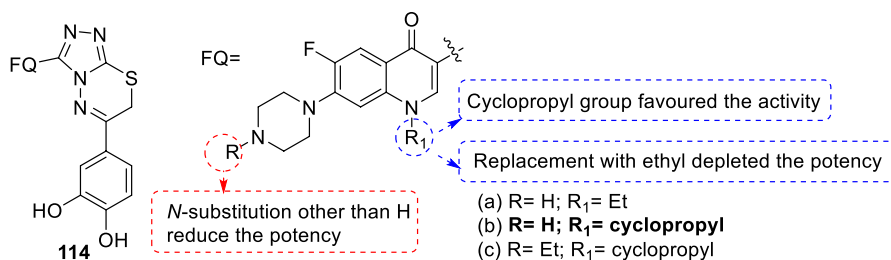**Fig. 29** SAR study of 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine derivatives as potent anticancer agents

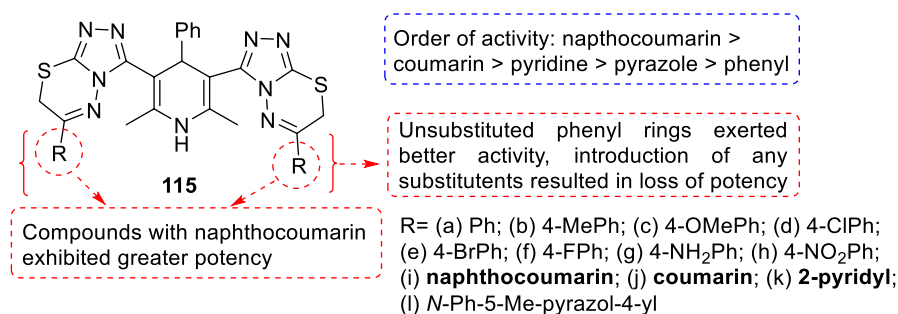having cyclopropyl substituted fluoroquinolones displayed better activity against L1210 cell lines, with an IC_{50} value of 0.14 μmol/L (Fig. 28).

Gomha et al. [86] explored bis-triazolothiadiazines (**115**) as potent anticancer agents against human hepatocellular carcinoma (HepG-2) cell lines using harmine as a reference. Bioassay results showed that compounds **115i–k** exhibited promising potency with IC_{50} value of 24.8, 28.3, and 31 μM, respectively. SAR studies demonstrated that compound substituted with naphthocoumarin ($IC_{50} = 24.8$) showed higher activity than a coumarin ($IC_{50} = 28.3$ μM), pyridine ($IC_{50} = 31$ μM) and pyrazole ($IC_{50} = 59.0$ μM) substituted analog (Fig. 29). Substitution of the phenyl ring with electron-donating (CH_3 or OCH_3) as well as electron-withdrawing groups (Cl, F or NO_2) diminished the cytotoxic activity compared with the unsubstituted phenyl group. In docking studies, potential interactions of **115i** and **115j** within the active site of DYRK1A are observed.

3.2 Antimicrobial Agents

Deohate et al. [87] accomplished the synthesis of 6,7-disubstituted phenyl-3-pyridin-4-yl-5*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines (**116**) and screened them for antimicrobial activity. Screening results indicated that compounds **116b**, **116e, f** showed highest inhibition against bacterial strains *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella typhi* (zone of inhibition = > 21 mm) and moderate
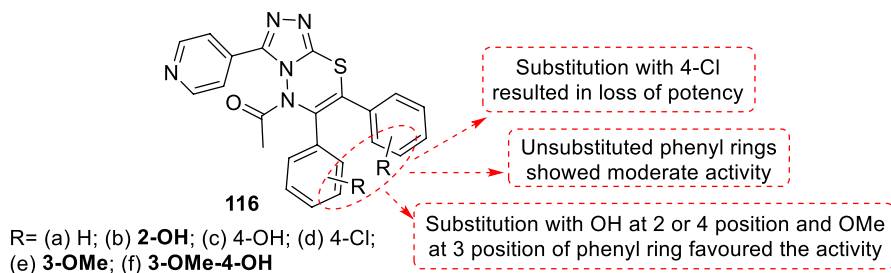
activity against *Aerobacter aerogenes* (zone of inhibition = 17–20 mm). Most of the tested compounds were found inactive against *Bacillus subtilis* (zone of inhibition = 13–16 mm). A SAR study showed that compounds containing electron donating substituents on the phenyl ring at position-6 and -7 of triazolothiadiazine enhanced the biological response markedly in comparison with electron-withdrawing groups, as depicted in Fig. 30.
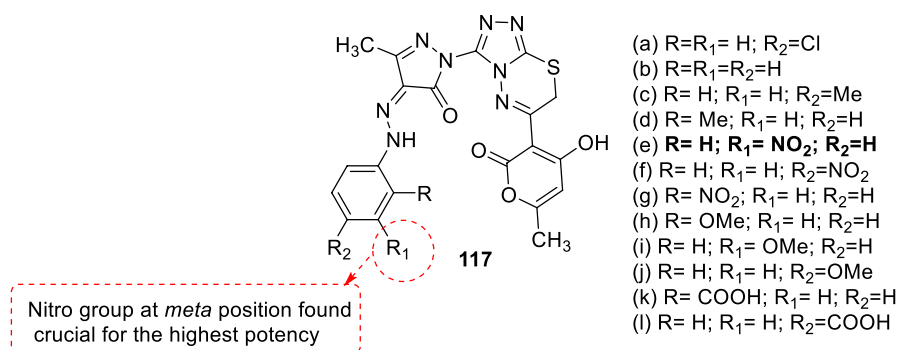
Penta et al. [88] synthesized pyrazolyl-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl)-2*H*-pyran-2-one derivatives (**117**) and evaluated their in vitro antibacterial activity against *S. aureus*, *B. subtilis*, *E. coli* and *Klebsiella pneumonia* and antifungal activity against *Candida albicans*. Among the tested compounds, compound **117e** showed better inhibition (zone of inhibition = 27–35 mm) than standard drugs (zone of inhibition = 25–32 mm). SAR study revealed that derivative containing electron-withdrawing group on phenyl ring exhibited most potent antimicrobial activity (Fig. 31).

Mekala et al. [89] reported indole-based 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine derivatives (**118**) and screened them for antifungal activity against *Aspergillus fumigatus*, *C. albicans*,*Trichophyton rubrum* and *Trichophyton mentagrophytes*. Amongst the tested compounds, compound **118c** was highly potent against *T. rubrum* and *T. mentagrophytes* (MIC = 3.12 μg ml^{-1}) while compound **118e** showed high activity against *C. albicans* (MIC = 3.12 μg ml^{-1}) even better than standard amphotericin-B (MIC = 6.25 μg ml^{-1}). SAR studies revealed that the electron-withdrawing group enhanced the biological impact (Fig. 32).

Jadhav et al. [90] synthesized a series of 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine derivatives (**119**) linked with chromeno[4,3-*b*]pyridin-5-one moiety and screened for antibacterial potency against *S. aureus*, *E. coli*, *B. cereus* and antifungal activity against *C. albicans* and *Aspergillus clavatus*. Compound **119c** showed significant antifungal effect against *C. albicans* (MIC = 5 μg ml^{-1}), comparable to that of standard ciprofloxacin (MIC = 5 μg ml^{-1}). Compound **119d** showed good inhibitory activity against the bacteria *B. cereus* (MIC = 5 μg ml^{-1}) and fungus *A. clavatus* (MIC = 5 μg ml^{-1} which was nearly the same as the standard ciprofloxacin and ketokonazole (MIC = 5 μg ml^{-1}. SAR studies revealed that compounds containing chloro or methoxy substituent displayed high potency (Fig. 33).

R= (a) H; (b) **2-OH**; (c) 4-OH; (d) 4-Cl; (e) **3-OMe**; (f) **3-OMe-4-OH**

116

Substitution with 4-Cl resulted in loss of potency

Unsubstituted phenyl rings showed moderate activity

Substitution with OH at 2 or 4 position and OMe at 3 position of phenyl ring favoured the activity

Fig. 30 SAR study and antimicrobial properties of 6,7-disubstituted phenyl-3-pyridin-4-yl-5*H*-[1,2,4] triazolo[3,4-*b*][1,3,4]thiadiazines

(a) R=R$_1$= H; R$_2$=Cl
(b) R=R$_1$=R$_2$=H
(c) R= H; R$_1$= H; R$_2$=Me
(d) R= Me; R$_1$= H; R$_2$=H
(e) **R= H; R$_1$= NO$_2$; R$_2$=H**
(f) R= H; R$_1$= H; R$_2$=NO$_2$
(g) R= NO$_2$; R$_1$= H; R$_2$=H
(h) R= OMe; R$_1$= H; R$_2$=H
(i) R= H; R$_1$= OMe; R$_2$=H
(j) R= H; R$_1$= H; R$_2$=OMe
(k) R= COOH; R$_1$= H; R$_2$=H
(l) R= H; R$_1$= H; R$_2$=COOH

Nitro group at *meta* position found crucial for the highest potency

Fig. 31 Structure and antimicrobial properties of pyrazolyl-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl)-2*H*-pyran-2-one derivatives

R= (a) H; (b) OMe; (c) **Cl**; (d) Br; (e) **NO$_2$**; (f) OH

Substitution with electron withdrawing groups (Cl, NO$_2$) improved the potency significantly
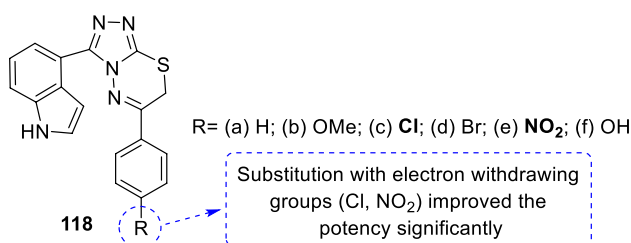
Fig. 32 SAR studies and antimicrobial properties of triazolothiadiazine analogs

System is essential to acheive high potency

Chloro and methoxy substituents showed good activity than unsubstituted ring

R= (a) Ph; (b) 4-BrPh; (c) **4-ClPh**; (d) **4-OMePh**; (e) 4-FPh
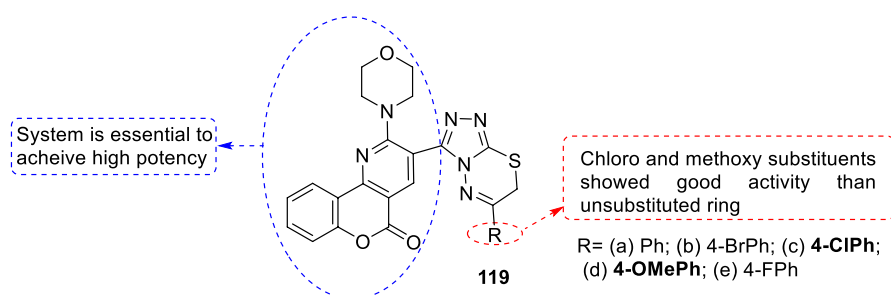
Fig. 33 SAR evaluation and antimicrobial properties of triazolothiadiazines containing chromeno[4,3-*b*]pyridin-5-one moiety

Iradyan et al. [91] carried out synthesis and antibacterial evaluation of 6-(2′-alkoxyphenyl)-3-aryl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines (**120**) against *S. aureus*, *Shigella flexneri* and *E. coli*. Of the screened compounds, **120a** and **120b** showed the most promising results (zone of inhibition = 15.6–23.6 mm). SAR studies showed that propoxy group at ortho-position of the phenyl ring was accountable for promoting the activity (Fig. 34).

Ghosh et al. [92] introduced a series of triazolothiadiazines (**121**) bearing substituted phenylquinolin-2-one moiety and evaluated them for their antibacterial

Fig. 34 SAR appraisal and antibacterial properties of triazolothiadiazines derivatives

Fig. 35 SAR and antimicrobial (inhibition zone) activities of triazolothiadiazine derivatives

activity against *Pseudomonas aeruginosa*, *E. coli*, *S. aureus*, and antifungal activity against *A. fumigatus*, *A. niger* and *C. albicans*. SAR study revealed that triazolothiadiazi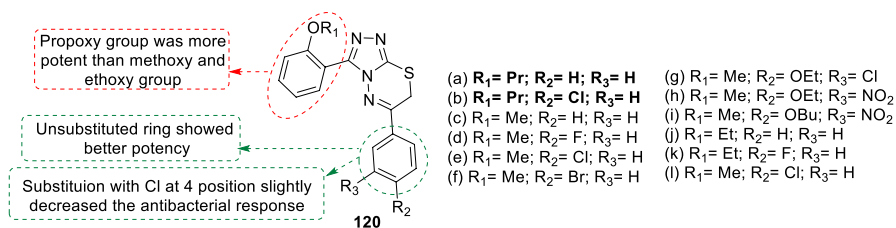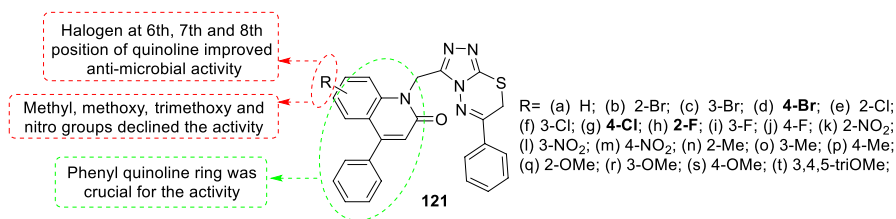ne derivatives bearing phenyl quinoline moiety possessed moderate to good antimicrobial activities and halogen substituted compounds **121d**, **121g** and **121h** showed the promising antifunfal activity against *C. albicans* with zones of inhibition of 20 mm, 23 mm and 19 mm, respectively (Fig. 35).

El-Sayed et al. [93] reported synthesis of 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines (**122**) bearing a 1,2,3-triazole moiety and screened them for antimicrobial activity against bacterial and fungal strains. Out of the tested series, the most potent compound **122b** exhibited moderate activity against bacterial strains of *E. coli*, *P. aeruginosa*, *S. aureus*, and *B. subtilis* (zone of inhibition = 14–18 mm) and fungal strains of *C. albicans*, and *Aspergillus flavus* (zone of inhibition = 17 and 19 mm) as compared with standard drugs. SAR study revealed the effect of different substituents on 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine scaffold for antimicrobial activity as depicted in Fig. 36.

Bhat et al. [45] reported the synthesis of 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine derivatives (**123**) and in vitro antifungal potential against various *Candida* species, namely *C. albicans*, *C. tropicalis*, *C. parapsilosis* and other *Candida* isolates (*Candida* sp. [HVS] 13,184, *Candida* sp. [HVS] 11,972, *Candida* sp. [HVS] 178, *Candida* sp. [urine] 300, *Candida* sp. [urine] 12,341, *Candida* sp. [urine] 12,485, *Candida* sp. [blood] 12,810). Among the screened derivatives, compounds **123a**, **123c**, **123e** and **123g** exhibited higher antifungal activity against some *Candida* species (MIC = 6.25–12.5 μg ml^{-1}) than the standard drug ketoconazole (MIC = 7–30 μg ml^{-1}). SAR studies indicated that *p*-chlorophenyl substitution on triazolothiadiazine ring positively influenced the anti-*Candidal* activity (Fig. 37).
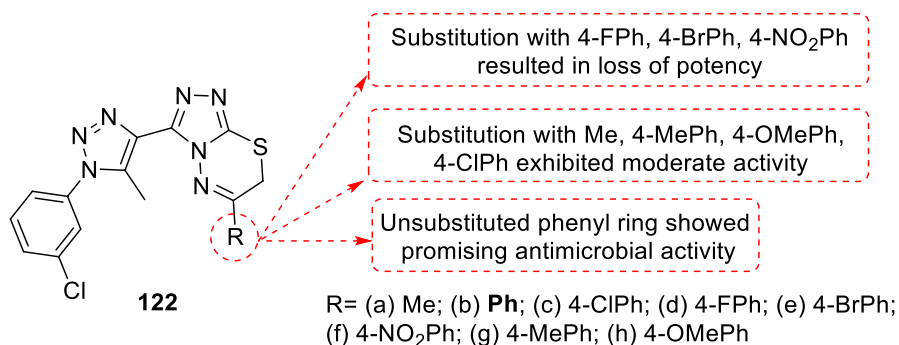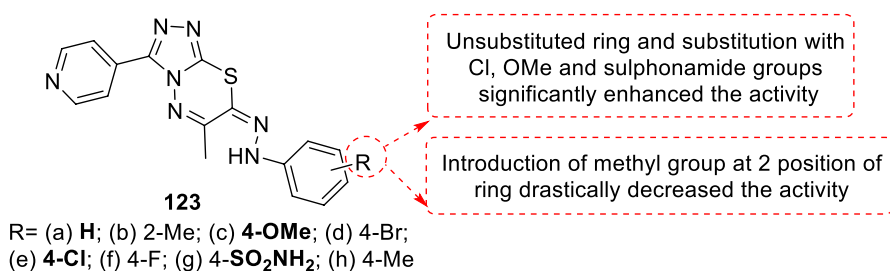
Fig. 36 SAR evaluation and antimicribial properties of triazolothiadiazine derivatives

Altintop et al. [94] synthesized new 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine derivatives (**124**) and screened for their in vitro anti-*Candidal* activity. Among all compounds, **124i** (R = cyclohexyl, R₁ = Cl) exhibited more potent activity against *C. albicans* (ATCC 90,028) with a MIC value of 0.03 mg ml⁻¹ in comparison to keto-conazole (MIC = 0.04 mg ml⁻¹). SAR studies revealed the significance of cyclohexyl group and *p*-chlorophenyl substituent on triazolothiadiazine ring for anti-*Candidal* activity (Fig. 38).

Prakash et al. [95] synthesized two series of dihydroindeno (**125**) and indeno-clubbed 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine derivatives (**126**) and evaluated their antimicrobial effects against *S. aureus, B. subtilis*, *E. coli*, *P. aeruginosa*, *A. niger*, *A. flavus* and *Penicillium species*. Bioassay results showed that dihydroindeno derivatives are more potent than indeno derivatives in terms of antimicrobial activity, and the tested compounds showed more potency towards Gram positive bacteria than towards Gram negative bacteria. Dihydroindeno compounds **125d–e, 125g–i** and indeno compounds **126d, 126g, 126j** exhibited noticeable antibacterial activities (zone of inhibition = 14.3–28.6 mm). In antifungal screening, compounds **125h**, **126g** and **126j** exhibited highest potential (mycelial growth inhibition = 51.1–68.8%) compared with standard drug fluconazole. A brief SAR study is presented in Fig. 39.

Badr et al. [96] accomplished the synthesis of 6-(4-substituted phenyl)-3-(5-nitrofuran-2-yl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine analogs (**127**) and screened their antibacterial potential against *S. aureus* and *E. coli*. Results

Fig. 37 SAR and anti-*Candidal* active triazolothiadiazines derivatives
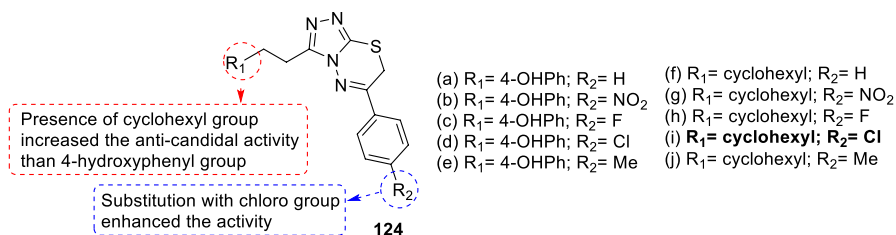
(a) R_1= 4-OHPh; R_2= H
(b) R_1= 4-OHPh; R_2= NO_2
(c) R_1= 4-OHPh; R_2= F
(d) R_1= 4-OHPh; R_2= Cl
(e) R_1= 4-OHPh; R_2= Me

(f) R_1= cyclohexyl; R_2= H
(g) R_1= cyclohexyl; R_2= NO_2
(h) R_1= cyclohexyl; R_2= F
(i) R_1= **cyclohexyl; R_2= Cl**
(j) R_1= cyclohexyl; R_2= Me

Presence of cyclohexyl group increased the anti-candidal activity than 4-hydroxyphenyl group

Substitution with chloro group enhanced the activity

124

Fig. 38 SAR appraisal and anti-*Candidal* activities of triazolothiadiazines derivatives

revealed that the tested compounds showed good activity against *S. aureus*. Compounds **127a** and **127b** were found to be as effective (MIC = 25 µg ml^{-1}) as the reference drug ampicillin (MIC = 25 µg ml^{-1}) against *S. aureus*. From the SAR, it was deduced that halogen groups on the aryl ring are essential for biological response (Fig. 40).

Sumangala et al. [97] synthesized 6-aryl-3-[4-(methylsulfonyl)benzyl]-7*H*-[1,2,4] triazolo[3,4-*b*][1,3,4]thiadiazines (**128**) and evaluated their antibacterial activity against *S. aureus*, *E. coli*, *P. aeruginosa*, *B. cereus* and antifungal activity against *C. albicans*. Compounds **128d**, **128i** and **128h** showed excellent antibacterial activity against *E. coli* (MIC = 3.125 mg ml^{-1}), *P. aeruginosa* (MIC = 3.125 mg ml^{-1}) and *B. cereus* (MIC = 3.125 mg ml^{-1}), respectively. Compounds **128b**, **128e**, **128f** and **128h** exhibited good antifungal activity (MIC = 1.562–3.125 mg ml^{-1}). A brief SAR of most active compounds is explained in Fig. 41.

Dihydroindeno derivatives were more potent than indeno derivatives

Substitution improves the activity significantly

(a) R= H; R_1= H
(b) R= H; R_1= Me
(c) R= H; R_1= Et
(d) R= H; R_1= Pr
(e) R= H; R_1= *i*-Pr
(f) R= H; R_1= Ph

(g) R= Me; R_1= H
(h) R= Me; R_1= Me
(i) R= Me; R_1= Et
(j) R= Me; R_1= Pr
(k) R= Me; R_1= *i*-Pr
(l) R= Me; R_1= Ph

125   **126**
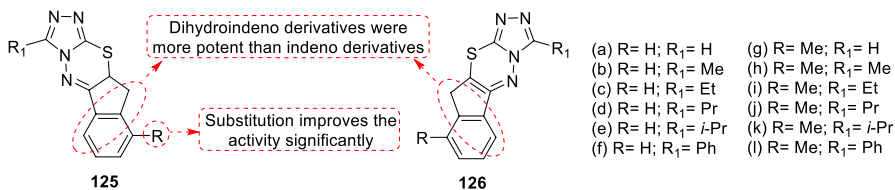
Fig. 39 SAR and activity values of dihydroindeno and indeno clubbed 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines

Halogen substituted phenyl group enhanced the antibacterial activity

Substitution with NO_2 or OMe group decreased the biological potential

127

R= (a) H; (b) **Cl**; (c) **Br**; (d) NO_2; (e) OMe

Fig. 40 SAR of 6-(4-substituted phenyl)-3-(5-nitrofuran-2-yl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines as antibacterial agents

Fig. 41 SAR and antimicrobial potential of 6-aryl-3-[4-(methylsulfonyl)benzyl]-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines

Fig. 42 SAR examination and antimicrobial screening results of 7*H*-7-alkoxy-3-alkyl/phenyl-6-aryl-*s*-triazolo[3,4-*b*][1,3,4]thiadiazines

Pundeer et al. [98] synthesized 7*H*-7-alkoxy-3-alkyl/phenyl-6-aryl-*s*-triazolo[3,4-*b*][1,3,4]thiadiazine derivatives (**129**) and tested for antimicrobial potency against *S. aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa*, *Saccharomyces cerevisiae* and *C. albicans*. Compounds **129f** 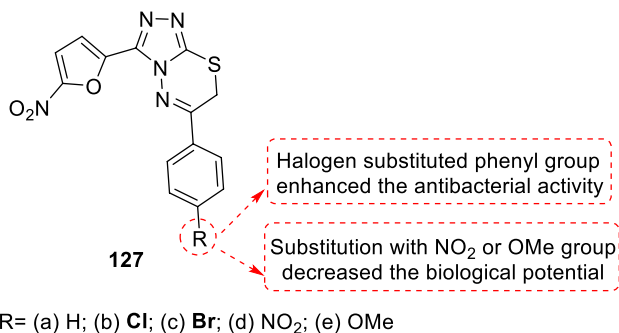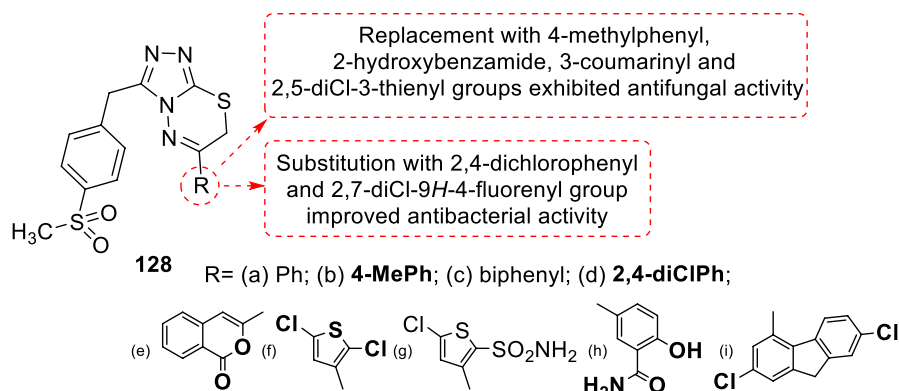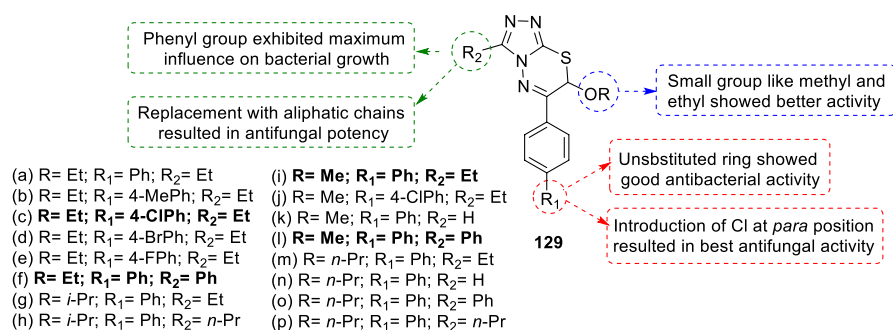and **129l** exhibited maximum inhibition against *S. aureus*, with MIC = 16 and 16 μg ml^{-1}, respectively, while compound **129i** generated good inhibition against *B. subtilis* with MIC = 32 μg ml^{-1}. In antifungal screening, compounds **129c**, **129f** and **129i** exhibited potent activity (MIC = 32–64 μg ml^{-1}) in comparison with the standard drug amphotericin-B (MIC = 100 μg ml^{-1}). A SAR study revealed the effect of substitution on the scaffold (Fig. 42).

Puthiyapurayil et al. [77] developed a novel 6-arylsubstituted-3-[2-(4-substituted phenyl)propan-2-yl]-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines (**130**) and evaluated antimicrobial potential against *E. coli*, *S. aureus*, *P. aeruginosa*, *B. subtilis*, *Rhizopus oryzae*, *A. fumigatus*, *A. niger* and *T. mentagrophytes.* Bioassay results revealed that, out of the evaluated derivatives, compounds **130e**, **130j** and **130m** showed equipotent antibacterial and antifungal activity to the standard streptomycin
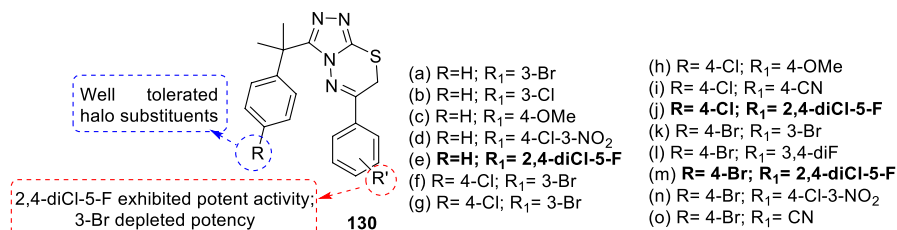
Fig. 43 SAR assesment and antimicrobial assay of 6-arylsubstituted-3-[2-(4-substitutedphenyl)propan-2-yl]-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines
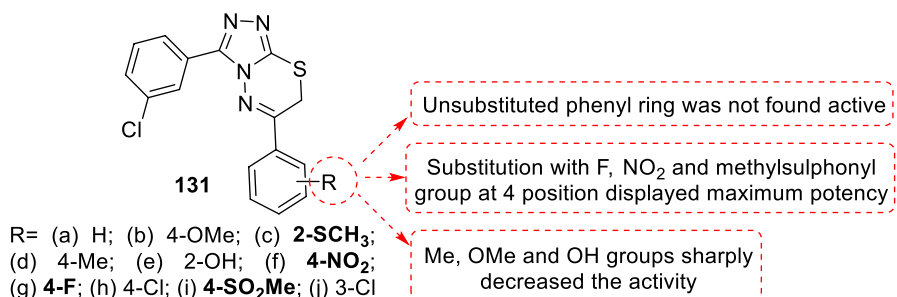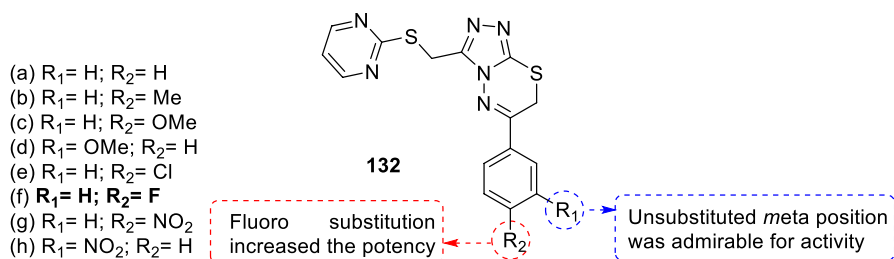

Fig. 44 SAR and antimicrobial studies of triazolothiadiazine derivatives

and amphotericin-B (MIC $= 6.25$ μg ml^{-1}). SAR studies explained that, due to their increased lipophilicity, highly electronegative substituents (F and Cl) facilitate hydrophobic interactions of the compounds with specific binding sites on either receptor or enzymes (Fig. 43).

Purohit et al. [99] reported a new series of 3-(3-chlorophenyl)-6-aryl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines (**131**) and evaluated their antimicrobial effects against *B. megaterium*, *S. aureus*, *E. coli*, *P. aeruginosa*, and *A. niger*. Among the tested series, compounds **131g** and **131i** showed significant activity against bacterial and fungal strains (zone of inhibition $= 18$–24 mm) while **131c** and **131f** exhibited promising antibacterial activity against *B. megaterium*, *P. aeruginosa* and *E. coli* (zone of inhibition $= 16$–20 mm). The pharmacological assessment and SAR of active compounds are given in Fig. 44.

Sim et al. [100] synthesized and screened pyrimidine linked 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines (**132**) for their antibacterial potential against *B. subtilis*, *B. cereus*, *S. aureus*, *P. aeruginosa*, *S. typhimurium*, and *E. coli*. Screening results indicated that compound having 4-flurophenyl ring **132f** demonstrated moderate activity against *P. aeruginosa* with MIC $= 62.5$ μg ml^{-1} (Fig. 45).

Almajan et al. [101] accomplished the synthesis of phenylsulfonylphenyl containing 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine derivatives (**133**) and assessed their in vitro antibacterial potential against the Gram-positive (*E. faecalis*, *S. aureus*, *S. epidermidis*, *B. cereus*) and Gram-negative (*A. baumanii*, *C. freundii*, *E. clocae*, *E. coli*, *P. aeruginosa*) bacterial strains through the broth dilution method.

(a) R₁= H; R₂= H
(b) R₁= H; R₂= Me
(c) R₁= H; R₂= OMe
(d) R₁= OMe; R₂= H
(e) R₁= H; R₂= Cl
(f) **R₁= H; R₂= F**
(g) R₁= H; R₂= NO₂
(h) R₁= NO₂; R₂= H

132

Fluoro substitution increased the potency

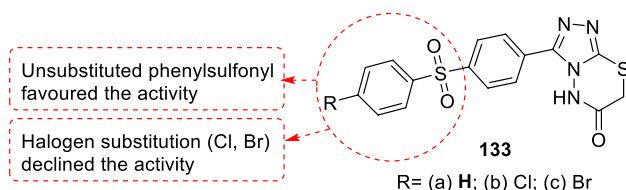Unsubstituted *meta* position was admirable for activity

Fig. 45 SAR appraisal and antibacterial studies of pyrimidine linked triazolothiadiazine derivatives

Compound **133a** was found to be most potent derivative against *S. epidermidis* with MIC $= 32$ μg ml^{-1} (Fig. 46). Bioassay results demonstrated that screened derivatives exerted better inhibition against Gram-positive bacterial strains than Gram-negative bacterial strains.

Reddy et al. [102] synthesized 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine derivatives (**134**) and evaluated their in vitro antibacterial activity. Among them, compound **134c** exhibited promising antibacterial activity against *E. coli, K. pneumoniae, S. dysentriae,* and *S. flexnei* with zone of inhibition value of 30 mm, 29 mm, 29 mm, and 27 mm, respectively, which was more active than standard neomysin (inhibition zone $= 20$ mm) and equipotent to streptomycin (inhibition zone $= 30$ mm) (Fig. 47).

Kumar et al. [103] prepared a series of 3-(4-isopropylthiazol-2-yl)-6-substituted phenyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines (**135**) and evaluated them for antimicrobial activity against *S. aureus*, *S. faecalis*, *B. subtilis*, *K. pneumoniae*, *E. coli* and *P. aeruginosa* and antifungal activity against *S. cerevisiae*, *C. tropicalis* and *A. niger*. Compound **135b** revealed significant antimicrobial potential against tested bacterial and fungal strains (MIC $= 8$, 4, 8, 16, 16, 8, 16, 31.25, 16 μg ml^{-1} against *S. aureus*, *S. faecalis*, *B. subtilis*, *K. pneumoniae*, *E. coli*, *P. aeruginosa*, *S. cerevisiae*, *C. tropicalis* and *A. niger*, respectively) (Fig. 48).

Indole clubbed derivatives of triazolothiadiazine (**136**) were evaluated for their antibacterial potential against *B. subtilis*, *B. cereus*, *S. aureus*, *M. luteus*, *E. coli*, *P. aeruginosa*, *S. typhimurium* and *P. vulgaris* by Sim et al. [104]. Compound **136d** was found to show significant inhibition against *B. subtilis*, *B. cereus* and *S. aureus* with MIC $= 3.91$ μg ml^{-1}. SAR studies revealed the importance of chlorine on indole moiety and *p*-methylphenyl group at C-6 position of triazolothiadiazine ring (Fig. 49).

Unsubstituted phenylsulfonyl favoured the activity

Halogen substitution (Cl, Br) declined the activity

133

R= (a) **H**; (b) Cl; (c) Br

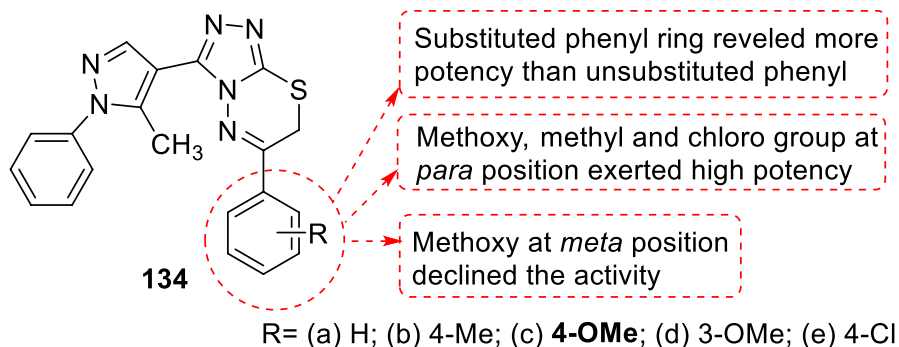**Fig. 46** Antibacterial evaluation and SAR of triazolothiadiazine derivatives

Fig. 47 SAR and antibacterial studies of triazolothiadiazine derivatives
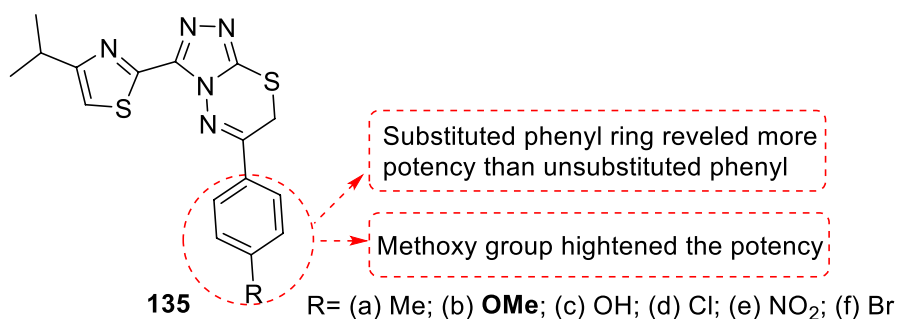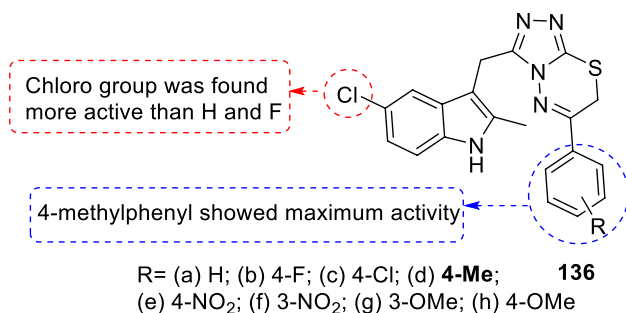

Fig. 48 SAR and antibacterial evaluation of isopropylthiazole clubbed derivatives of triazolothiadiazine

3.3 Analgesic and Anti-inflammatory Agents

Puthiyapurayil et al. [105] introduced amide linked 3,6-disubstituted-7*H*-1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine derivatives (**137**) and screened them for their in vivo analgesic and anti-inflammatory potential using acetic acid-induced writhing test and carregeenan-induced rat hind paw edema, respectively. From the tested series, compounds **137a**, **137b** and **137c** exhibited potent and consistent analgesic activity in both 100 and 200 mg kg^{-1} po^{-1} doses with less ulcerogenic risk (inhibition = 28–43%) as well as excellent anti-inflammatory activity (at a dose of 50 mg kg^{-1}) (edema inhibition = 38–61%). SAR studies demonstrated that derivatives with *N*-methyl-[4-(trifluoromethyl)phenyl]pyrazole moiety at the position-3 of the fused ring are more potent than the other derivatives bearing [1-(4-chlorophenyl)-1-methylethyl] as shown in Fig. 50. Compound **137b** also displayed promising cytotoxic effect against human breast cancer (MCF-7) cell line with IC$_{50}$ value 14.24 μM.

Shehry et al. [106] reported synthesis and anti-inflammatory activity of 3-(2,4-dichlorophenoxy)methyl)-1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines (**138**). Compounds **138a** and **138b** exhibited potent anti-inflammatory activity (edema inhibition = 59–60% at 3 h). The percent change in paw volume compared with base line measurement was recorded after interval of 1 h. SAR studies demonstrated that

R= (a) H; (b) 4-F; (c) 4-Cl; (d) **4-Me**;   **136**
(e) 4-NO$_2$; (f) 3-NO$_2$; (g) 3-OMe; (h) 4-OMe

Fig. 49 SAR and antibacterial studies of indole clubbed triazolothiadiazines as potential antibacterial agents

R= (a) **4-Cl**; (b) **4-OMe**; (c) **2-F**; (d) NHCH$_3$
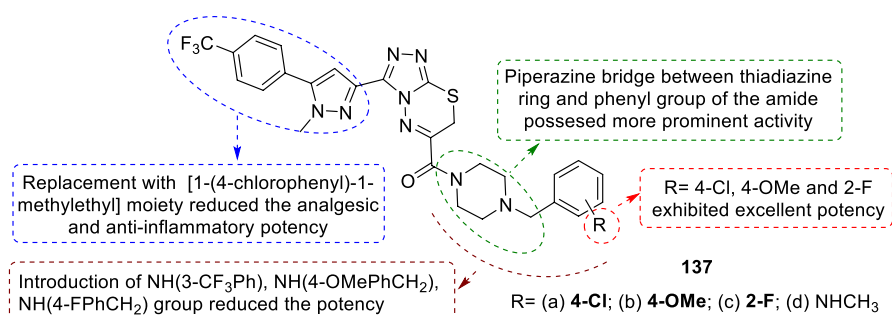
Fig. 50 SAR assessment and pharmacological evaluation results of triazolothiadiazines derivatives

2,4-dichlorophenoxy at the 3-position of triazolothidiazine ring was found crucial for biological effect (Fig. 51).

Hussein et al. [107] synthesized and evaluated 4-substituted phenyl-3-(pyridin-4-yl)-7H-(1,2,4)triazolo[3,4-b]-[1,3,4]thiadiazine derivatives (**139**) for their analgesic and anti-inflammatory effects. Among them, compounds **139d**, **139e** and **139f** (0.03 mmol kg^{-1}) displayed maximum anti-inflammatory effect at 5 h, with inflammatory inhibition rates of 75% and 84.85 and 75.31%, respectively. Moreover, compounds **139d–f** (0.02 mmol kg^{-1}) exhibited potent analgesic effects with 79–145% activity compared to indomethacinin at the 5 h interval. Figure 52 illustrates a brief SAR and activity profile of most active compounds.

Koksal et al. [17] reported a series of 3,6-diaryl-1,2,4-triazolo[3,4-b][1,3,4]thia-diazines (**140**) for their anti-inflammatory activity. The outcome showed that compounds **140c–h** and **140j** were found to have better inhibitory potency (50.2–62.8%) at different time interval with less ulcerogenic risk than the standard indomethacin (49.2% in 120 min, 54.9% in 240 min). SAR studies were carried out to determine the active functional groups at position-6 of triazolthiadiazine ring in order to enhance potency (Fig. 53).

Naveena et al. [108] reported the synthesis and anti-inflammatory activity evaluation of novel 6-substituted-3-(2-(aryloxymethyl)phenyl)[1,2,4]triazolo[3,4-b][1,3,4] thiadiazine derivatives (**141**). Bioassay results showed that the tested compounds
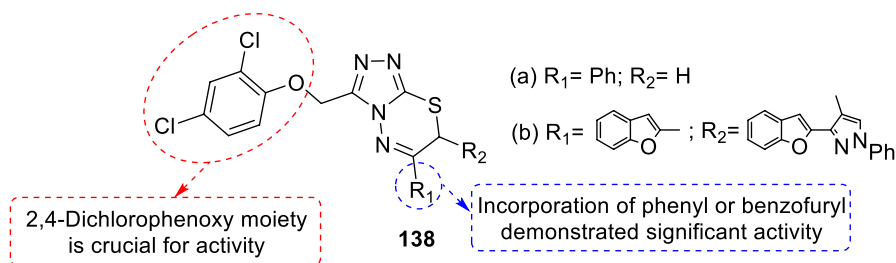
Fig. 51 SAR study and anti-inflammatory potential of 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine derivatives
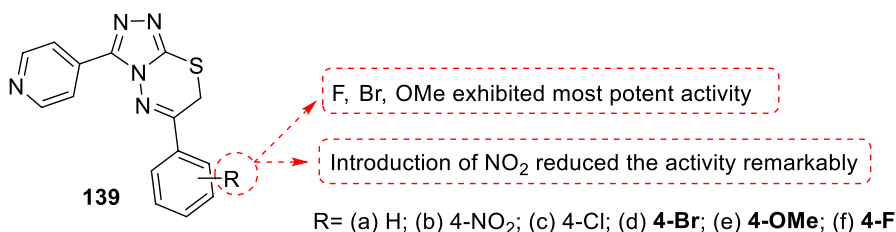
Fig. 52 SAR and analgesic/anti-inflammatory evaluation of triazolothiadiazines derivatives
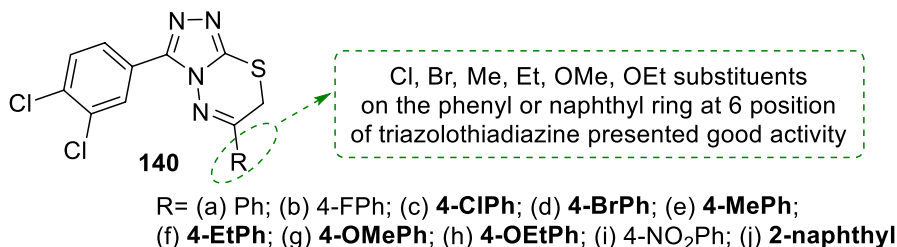
Fig. 53 SAR study and anti-inflammatory activity of 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine derivatives

displayed anti-inflammatory activity ranging from 40.52% to 70.88%. Compounds **141a–e** exhibited significant anti-inflammatory activity with 68.65–70.88% inhibition. A SAR study demonstrated that substitution with 5-chloro-2-sulfanamido-3-thienyl and 4-nitrophenyl groups at position-6 of triazolothiadiazine scaffold enhanced the anti-inflammatory activity (Fig. 54).

3.4 Antioxidant Agents

Ranganatha et al. [109] synthesized diarylmethanone linked 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine derivatives (**142**) and evaluated their in vitro antioxidant potential by DPPH, nitric oxide and hydrogen peroxide scavenging assay. Among the tested series, compounds **142b**, **142c** and **142d** exhibited significant antioxidant effect in DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) test with the IC_{50} values of 18.33,
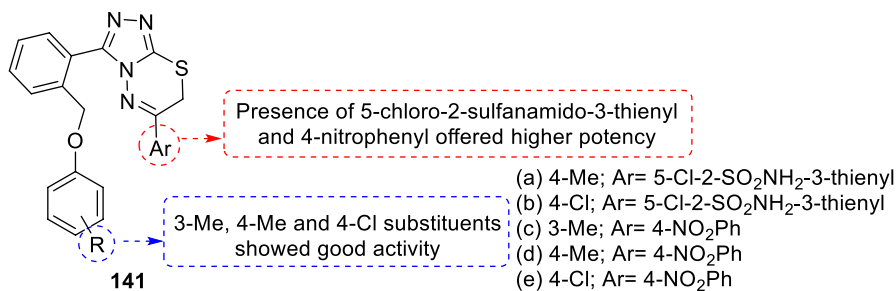
Fig. 54 SAR studies and anti-inflammatory data of active triazolothiadiazines derivatives

16.32, 16.55 μg ml^{-1}, respectively, in comparison with standard ascorbic acid (IC$_{50}$ = 14.98 μg ml^{-1}). SAR studies were carried out to determine the active functional groups in order to enhance the potency (Fig. 55).

Patrao et al. [110] synthesized and screened antioxidant activity of a cluster of α and β-naphthoxy methyl linked 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine derivatives (**143**) using the DPPH assay. The results indicated that, among the tested derivatives, compound **143b** exhibited highest antioxidant potential, with inhibition of 60% at a concentration of 100 μg ml^{-1}. SAR studies demonstrated that derivatives linked with α-naphthoxy group exhibited enhanced activity in comparison with β-naphthoxy derivatives (Fig. 56).

Kaushik et al. [111] prepared 3-substituted phenyl-6-phenyl-7*H*-[1,2,4] triazolo[3,4-b][1,3,4]thiadiazine derivatives (**144**) and screened their antioxidant activity on the basis of the scavenging activity of the stable DPPH free radical. Compounds **144a** and **144e** possess significant antioxidant potential with IC$_{50}$ values of 17.27 and 17.05 μg ml^{-1}, respectively. SAR studies revealed that antioxidant activity of compounds may depend on the basic skeleton of the molecule as well as on the nature of substituents (Fig. 57).

Shakir et al. [112] reported synthesis and antioxidant activity evaluation of 6-amino-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl derivatives (**145**) bearing 2,6-dimethoxy-4-(methoxymethyl)phenol moiety by DPPH and ferric reducing antioxidant power (FRAP) assays. Among the screened compounds, compound **145b**
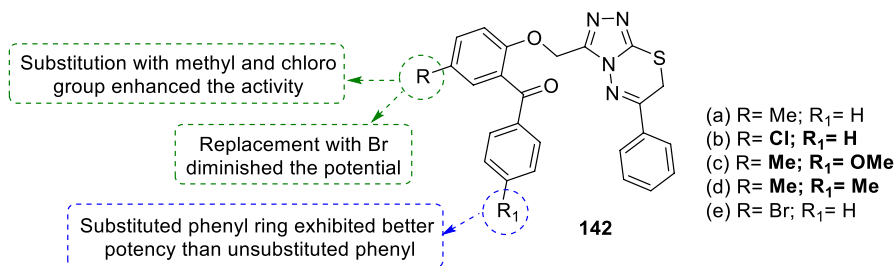
Fig. 55 SAR and antioxidant potential of active triazolothiadiazines derivatives using the DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) method
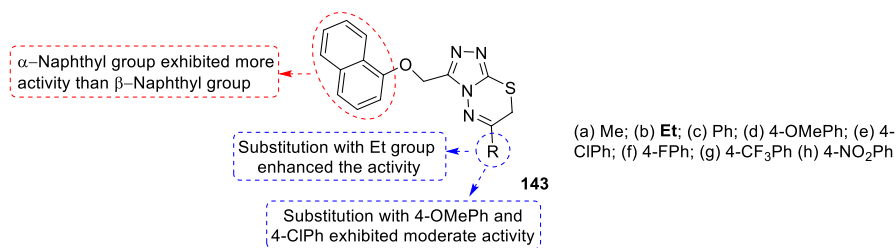
Fig. 56 SAR and radical scavenging activity of triazolothiadiazines derivatives

exhibited remarkable antioxidant ability in both assays (DPPH inhibition = 92%; IC_{50} = 56 μg ml^{-1} which is higher than standard butylated hydroxytoluene (BHT) (DPPH inhibition = 66%; IC_{50} = 78 μg ml^{-1}). SAR studies revealed that compounds **145a–c** exhibited higher antioxidant potential with inhibition in the range 79–83% due to presence of thiourea moiety than compounds **145d–f** containing amide group with 68–75% inhibition (Fig. 58).

Sathyanarayana et al. [113] designed and synthesized benzimidazole linked 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine derivatives (**146**) and assessed their in vitro antioxidant potential using DPPH, nitric oxide and superoxide radical scavenging methods. Among them, compound **146c** showed more activity in both DPPH and nitric oxide radical assay, with IC_{50} values of 13.57 and 18.55 μg ml^{-1} compared with standard (IC_{50} = 23.75 and 23.14 μg ml^{-1}, respectively). SAR studies demonstrated that derivatives substituted with electron-donating groups exhibited enhanced activity in comparison with electron-withdrawing groups (Fig. 59).

3.5 Antiviral Agents

Pavurala et al. [114] synthesized a series of bis coumarinyl bis triazolothiadiazinyl ethane derivatives (**147**) and screened their antiviral activity against a broad spectrum of RNA and DNA viruses (herpes simplex virus-1 (KOS), herpes simplex virus-2 (G), vaccinia virus, vesicular stomatitis virus, herpes simplex virus-1TKKOSACV^r, adenovirus-2, vesicular stomatitis virus, coxsackie virus

R= (a) **H**; (b) 2-NH$_2$-4-Cl; (c) 4-F-3-NO$_2$; (d) 2,5-diOMe; (e) **4-CH$_2$NH$_2$**; (f) 4-NO$_2$-3-OH; (g) 2-NH$_2$-4-I; (h) 2-I; (i) 3-Cl-5-F
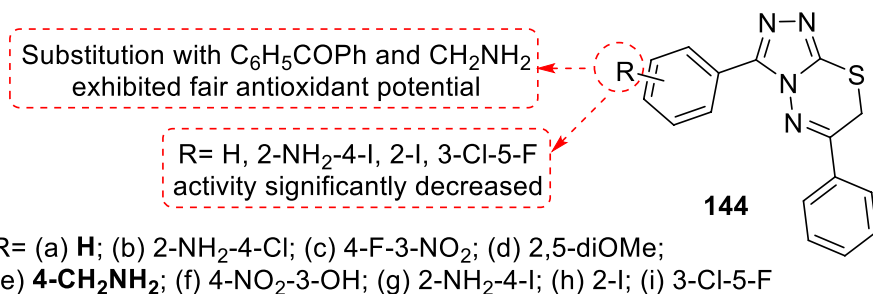
Fig. 57 SAR and antioxidant activities of triazolothiadiazines derivatives
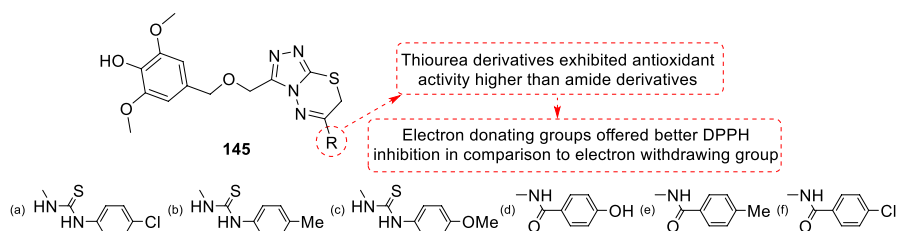
Fig. 58 SAR and antioxidant profile of active triazolothiadiazines derivatives
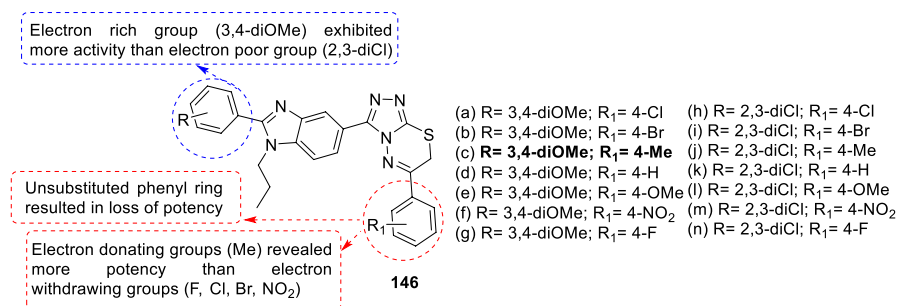
Fig. 59 SAR and antioxidant studies of triazolothiadiazines derivatives

B4, respiratory syncytial virus, para-influenza-3 virus, reovirus-1, sindbis virus, punta torovirus, feline coronavirus (FIPV), feline herpes virus, influenza A H1N1 subtype, influenza A H3N2 subtype, and influenza B) in HEL, HeLa, Vero, and CRFK cell medium. Out of 11 tested compounds, **147k** exhibited good activity against influenza A virus. In a cytopathic effect (CPE) reduction assay, compound **147k** exerted inhibitory effects against influenza virus infected Madin–Darby canine kidney (MDCK) cells, with EC_{50} values of 20–72 μM. Docking results revealed that compound **147k** (MoleDock score $= -173$ kcal mol^{-1}) inhibits the H1N1 neuraminidase enzyme via hydrogen bonding, electrophilic and hydrophobic interactions in the catalytic unit of H1N1 neuraminidase (Fig. 60). SAR studies revealed that compounds having an aromatic ring on a coumarin nucleus exhibit better antiviral activity than other substituents such as chloro, bromo or methoxy substituents.

Pandey et al. [50] introduced a series of 5-[(3′-aralkyl amido/imidoalkyl) phenyl]-1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines (**148**) and screened their in vitro antiviral potential against two animal viruses, namely Japanese encephalitis virus (JEV) strain P20778 and Herpes simplex virus-1 (HSV-1) strain 753,166. Screening results revealed that compound **148c**, with a 2-phenyl-3-methyl-quinazolin-(3*H*)-4-one group, is mainly responsible for exerting antiviral activity against JEV ($EC_{50} = 7.8$ μg ml^{-1} inhibition $= 50\%$; therapeutic index $= 32$) while compounds **148a**, **148b** and **148d** containing phthalimido, phthalimidomethyl, and nicotinamido substituents, respectively, exhibited weak activity (10–20%) against JEV and HSV-I (Fig. 61).
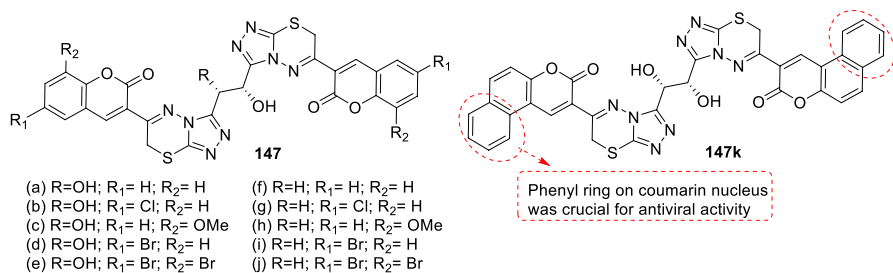
Fig. 60 Anti-influenza viral activity of bis coumarinyl bis triazolothiadiazinyl ethane derivatives

Khan et al. [115] synthesized 6-aryl-3-(furan-2-yl)-7*H*-[1,2,4]triazolo[3,4-*b*] [1,3,4]thiadiazine derivatives (**149**) and assessed their in vitro anti-HIV-1 (strain III$_B$) and anti-HIV-2 (strain ROD) activity by monitoring the inhibition of the virus-induced cytopathic effect in MT-4 cells. Bioassay results indicated that compound **149f** exerted inhibitory effects against replication of human immunodeficiency virus (HIV)-1 and -2 in cell cultures with EC$_{50}$ of >0.67 μm, but no selectivity was witnessed (SI < 1) (Fig. 62). Compound **149 g** with a nitro group at 4 position of the phenyl ring exhibited less anti-HIV activity (EC$_{50}$ = > 83.20 μm).

3.6 Enzyme Inhibitors

SitaRam et al. [116] synthesized 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine derivatives (**150**) containing benzenesulfonamide moieties and screened their inhibitory potential against four isomers of the α-carbonic anhydrases (CAs) comprising hCAs I and II (cytosolic, ubiquitous isozymes) and hCAs IX and XII (transmembrane, tumor-associated isozymes). Bioassay results revealed that compound **150a** exhibited excellent inhibitory potential against all four isozymes (K_i = 3.0–89 nM), **150b** inhibited isozymes hCA I, IX and XII (K_i = 84, 6.5 and 3.8 nM, respectively) while **150c** inhibited isozymes hCA IX and XII (K_i = 25 and 18 nM, respectively).
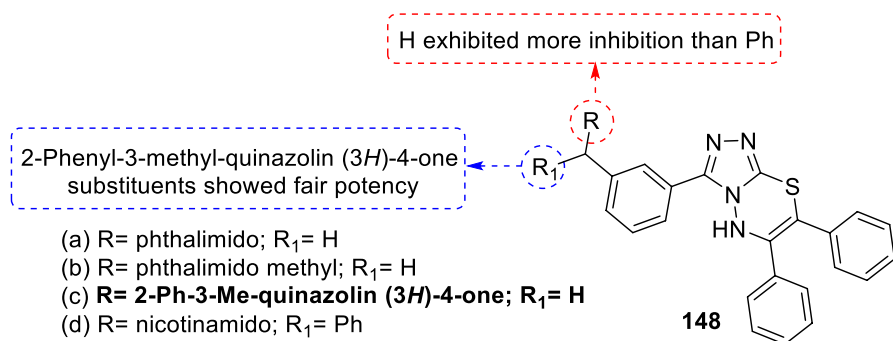
Fig. 61 Antiviral activity of 5-[(3′-aralkyl amido/imidoalkyl) phenyl]-1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines

R= (a) 4-OMePh; (b) 4-ClPh; (c) 4-FPh; (d) 4-MePh;
(e) biphenyl; (f) **1-naphthyl**; (g) **4-NO$_2$Ph**; (h) 3,4-diClPh
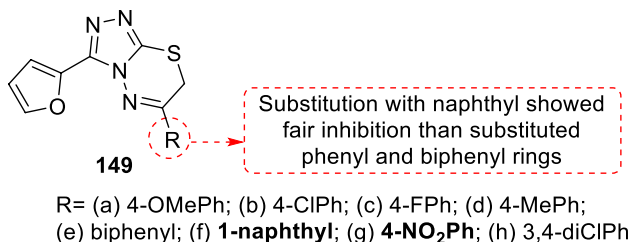
Fig. 62 SAR estimation and anti-HIV activity of furan linked triazolothiadiazines derivatives

Compounds **150d–e** selectively inhibited hCA IX ($K_i = 21$–25 nM) even better than the standard drug acetazolamide ($K_i = 25$ nM). SAR revealed the effect of different substituents on 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine for hCA inhibition (Fig. 63).

Alafeefy et al. [117] introduced a series of benzenesulfonamides incorporating aroylhydrazone-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazinyl group (**151**) and examined their inhibitory potential against four CAs, the human (h) isoforms hCA I, II, IX and XII. Bioassay results revealed that compound **151a** presented significant inhibitory effects against hCA I ($K_i = 86.4$ nM) (threefold more effective than the standard acetazolamide; $K_i = 250$ nM)) and hCA XII ($K_i = 4.3$ nM) while **151b** exhibited inhibition against hCA II and hCA XII ($K_i = 0.93$ and 0.85 nM, respectively) (Fig. 64).

Khan et al. [62] screened 3-(pyridin-4-yl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4] thiadiazine derivatives (**152**) for cholinesterase inhibition against electric eel acetylcholinesterase (EeAChE) and horse serum butyrylcholinesterase (hBChE) as well as for alkaline phosphatase inhibition. Results indicated that compound **152f** (IC$_{50}$ = 13.66 ± 0.31 μM) exhibited remarkable inhibition against EeAChE, while compounds **152g** (IC$_{50}$ = 16.6 ± 2.72 μM) and **152h** (IC$_{50}$ = 12.2 ± 1.67 μM) showed greater inhibitory potential against BChE more than the standard drug neostigmine. Compound **152h** also exhibited significant inhibitory effect on alkaline phosphatase with IC$_{50}$ = 1.32 ± 0.20 μM. SAR studies revealed the role of different functional groups on the triazolothiadiazine nucleus as shown in Fig. 65. In another work, evaluation of 3-(pyridin-3-yl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines (Fig. 65) for cholinesterase inhibitory activity by Khan et al. [63] revealed that compound **153g**

R= (a) **Br**; (b) **OMe**; (c) **Me**;
(d) **F**; (e) **H**; (f) Cl; (g) NO$_2$
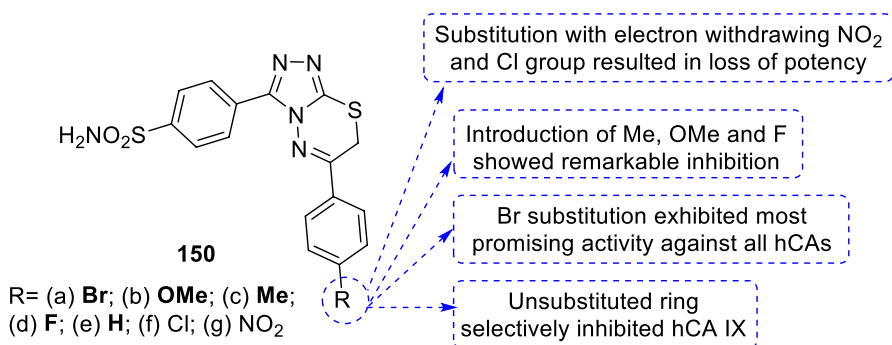
Fig. 63 SAR and carbonic anhydrases inhibition of 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine derivatives
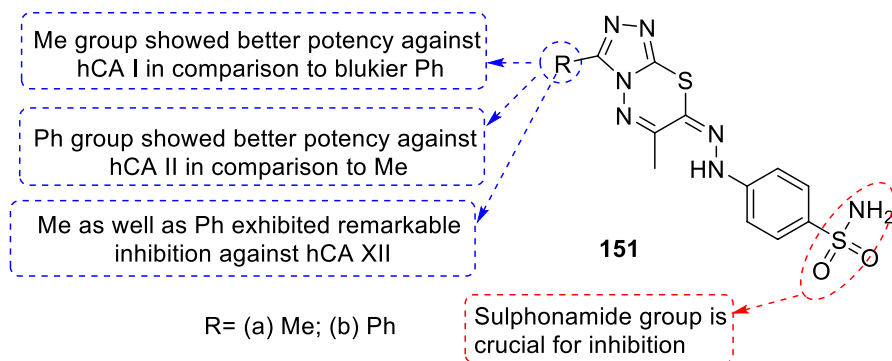
Fig. 64 SAR and pharmacokinetics of triazolothiadiazine derivatives as carbonic anhydrase inhibitors

($IC_{50} = 15.1 \pm 0.213$ μM) and **153h** ($IC_{50} = 1.09 \pm 0.156$ μM) exerted strong inhibitory effects on EeAChE and hBChE, respectively.

Ozil et al. [118] synthesized triazole-linked bis([1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine) derivatives (**154**) and assessed anti-lipase activity with regard to pancreatic lipase inhibition. Among them, compounds **154a–c** showed considerable anti-lipase activity at various concentrations (inhibition = 90–94%; $IC_{50} = 0.11$–0.42 μg ml^{-1}). SAR studies identified the significance of R groups on triazolothiadiazine ring for inhibition of pancreatic lipase (Fig. 66).

Recently, Çevik et al. [119] introduced benzimidazole-triazolothiadiazine derivatives (**155**) as aromatase inhibitors ($IC_{50} = 0.037 \pm 0.001$ μM to 1.345 ± 0.061) using fluorimetric assay kit with letrozole as the reference drug. Among tested compounds, compound **155g** was found to be most promising inhibitor against aromatase enzyme ($IC_{50} = 0.037$ μM), which was slightly less potent than standard drug letrozole ($IC_{50} = 0.024$ μM) (Fig. 67).

A series of 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines (**156**) was explored as dual inhibitors of cholinesterase (ChE) and monoamine oxidase (MAO) by Khan et al. [120]. Bioassay results revealed that compounds **156e** and **156h** exhibited high selectivity towards EeAChE, with IC_{50} values of 0.065 and 0.075 μM, respectively, which is around 250- and 218-fold stronger inhibition than the standard drug neostigmine ($IC_{50} = 16.3$ μM). Compound **156c** showed ~330-fold more inhibition
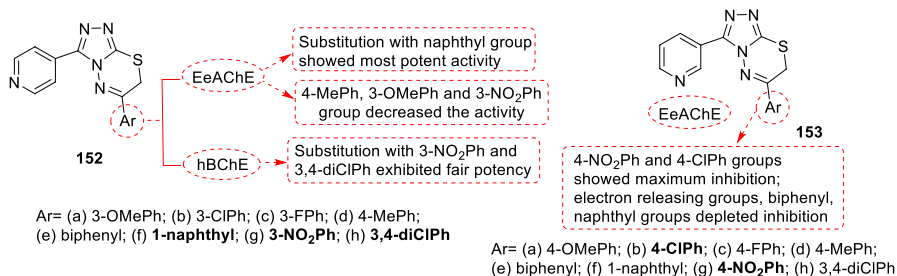
Fig. 65 SAR, cholinesterase and alkaline phosphatase inhibitory activities of triazolothiadiazines
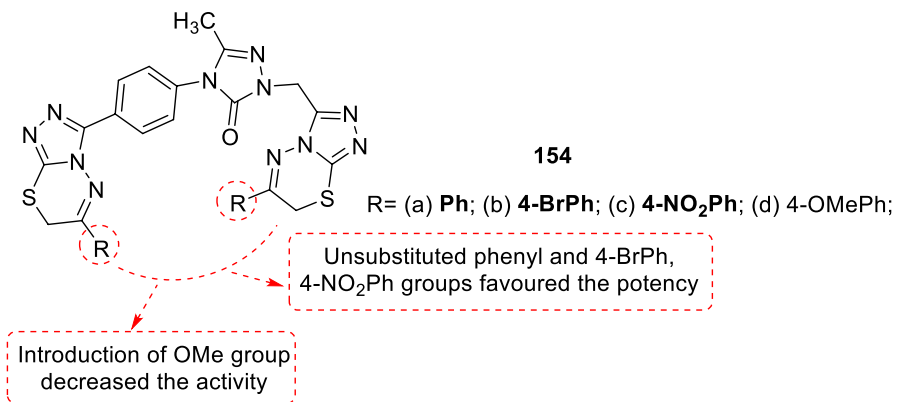
Fig. 66 SAR and anti-lipase activity of triazolothiadiazine derivatives
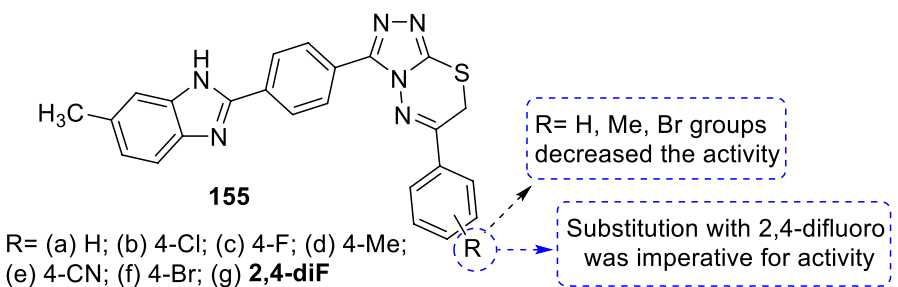
Fig. 67 SAR analysis and effect of benzimidazole linked triazolothiadiazine derivatives as aromatase inhibitors

than the standard drug clorgyline (IC$_{50}$ = 3.64 μM) towards MAO-A, with IC$_{50}$ values of 0.011 μM, while compound **156n** exhibited remarkable selectivity for MAO-B (IC$_{50}$ = 0.08 μM). SAR revealed the effect of different substituents on 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine on ChE inhibition and AChE/ BChE selectivity (Fig. 68).

Baeeri et al. [121] synthesized 3,6-diaryl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines (**157**) as potential phosphodiesterase-4 inhibitors. Among the tested derivatives, compound **157d** exerted remarkable specificity for PDE-4 by enhancing the concentration of cAMP (ED$_{50}$ = 13 μM), however the level of cGMP remains unaffected (ED$_{50}$ = 49.8 μM) (Fig. 69). Docking studies also validated the selective binding of these compounds in cAMP-specific PDE enzymes binding site.

3.7 Antitubercular Agents

Li et al. [122] reported new analogs of 3,6-disubstituted 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine (**158**) and examined their antitubercular activities against *Mycobacterium tuberculosis* (Mtb) H37Rv, multi-drug-resistant (MDR)-TB
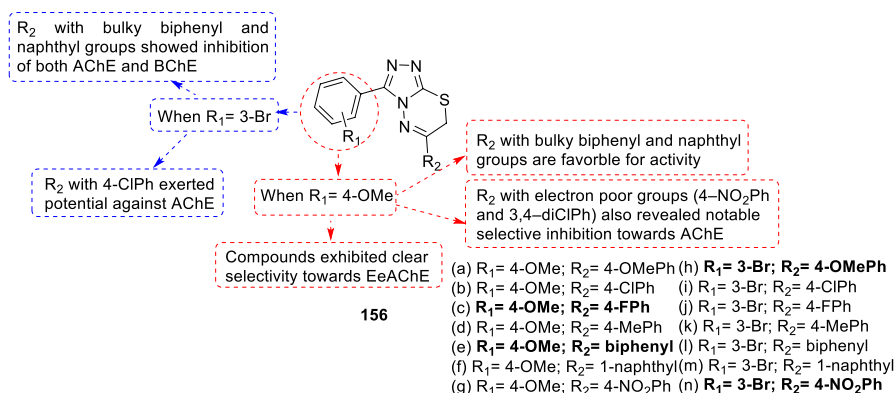
Fig. 68 SAR and exploration of triazolothiadiazine derivatives as cholinesterase (ChE) and monoamine oxidase (MAO) inhibitors

R= (a) 2-OMe; (b) 3-OMe; (c) 4-Me; (d) **H**   **157**
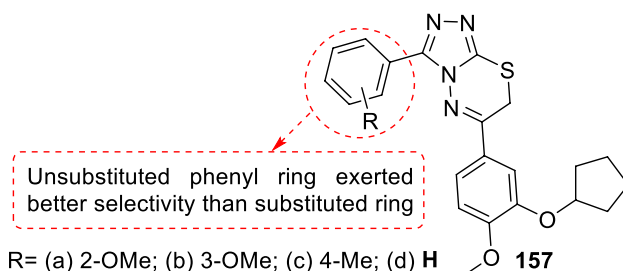
Fig. 69 SAR and evaluation of triazolothiadiazine derivatives as phosphodiesterase-4 inhibitors

and RDRTB (rifampin-resistant strains) by the BacT/ALERT 3D liquid culture technology using rifampin and isoniazid as the standard drugs. According to the results of antitubercular determination, compounds **158a**, **158i**, **158k**, **158r**, **158t** and **158o** exhibited most potent activity (MIC = 0.25–4 μg ml^{-1}; IC$_{50}$ = 28–252 μg ml^{-1}). A SAR study was carried out to correlate the effect on activity of aromatic or hetero-aromatic substituents at the 3- and 6-position of triazolothiadiazine, which is presented in Fig. 70.

Kincaid et al. [123] identified 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine derivatives (**159**) as antimycobacterial agents via inhibition of uridine 5′-diphosphate-galactopyranose mutase (UGM) by means of virtual screening (docking and X-ray crystallography studies). Bioassay outcomes showed that compounds **159g–j** (K_d = 5–15 μM; inhibition = 97–100%) possessed the most promising activity a,nd compound **159j** fully blocked the *K. pneumoniae* UGM activity at 50 μM. These derivatives also contributed antimicrobial potency against Mtb and *Mycobacterium smegmatis*. SAR studies of the derivatives revealed that compounds with larger or more polarizable phenyl ring on the position-6 and larger side-chains at position-3 of triazolothiadiazine ring exhibited effective inhibition (Fig. 71). Docking and X-ray structure showed consistency with the observed SAR.
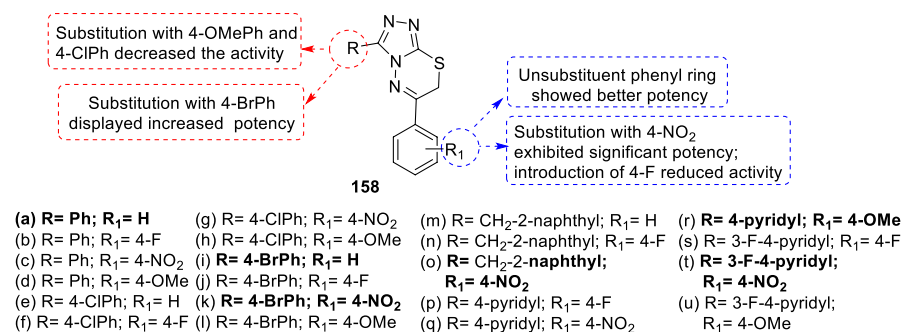
Fig. 70 SAR and antitubercular activity of 3,6-disubstituted 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine derivatives

(a) R= Ph; R$_1$= H	(g) R= 4-ClPh; R$_1$= 4-NO$_2$	(m) R= CH$_2$-2-naphthyl; R$_1$= H	(r) **R= 4-pyridyl; R$_1$= 4-OMe**
(b) R= Ph; R$_1$= 4-F	(h) R= 4-ClPh; R$_1$= 4-OMe	(n) R= CH$_2$-2-naphthyl; R$_1$= 4-F	(s) R= 3-F-4-pyridyl; R$_1$= 4-F
(c) R= Ph; R$_1$= 4-NO$_2$	(i) **R= 4-BrPh; R$_1$= H**	(o) **R= CH$_2$-2-naphthyl;**	(t) **R= 3-F-4-pyridyl;**
(d) R= Ph; R$_1$= 4-OMe	(j) R= 4-BrPh; R$_1$= 4-F	**R$_1$= 4-NO$_2$**	**R$_1$= 4-NO$_2$**
(e) R= 4-ClPh; R$_1$= H	(k) **R= 4-BrPh; R$_1$= 4-NO$_2$**	(p) R= 4-pyridyl; R$_1$= 4-F	(u) R= 3-F-4-pyridyl;
(f) R= 4-ClPh; R$_1$= 4-F	(l) R= 4-BrPh; R$_1$= 4-OMe	(q) R= 4-pyridyl; R$_1$= 4-NO$_2$	R$_1$= 4-OMe

4 Rational Design Based on SAR of Triazolothiadiazine Derivatives

The present review captures the diverse biological activities exhibited by triazolothiadiazines reported in the literature. After careful analysis, general structural features have been identified that can be used in the design and development of novel triazolothiadiazine deivatives having clinical efficiency. Unfused 5-membered triazole ring systems were found to be biologically inactive; however, fusion of the 5-membered triazole ring with 6-membered thiadiazine ring is important for bioactivity [59, 84]. Unsaturation in the 6-membered thiadiazine ring fueled bioactivity, in comparison with saturated derivatives [84]. At position-3 of the triazole ring, aromatic substitution connected through a spacer (0–3 atoms) exhibited better activity. Aromatic substituents at position-6 of the triazolothiadiazine ring displayed improved potency over the aliphatic analogs. Halogen-substituted aryl groups, specifically chloro-substituted aryl results in better activity than electron-donating groups, as aliphatic substitution bring down the activity. Substitution at position-7 of the triazolothiadiazine ring can be important for activity, but is not essential. Figure 72 summarizes the structural modifications in terms of different substituents at position-3, 6 and 7 (R$_1$, R$_2$ and R$_3$) with respect to various biological activities, namely anticancer, antiviral, antimicrobial, antioxidant, antitubercular, enzyme inhibitiors, analgesic and anti-inflammatory activities.

(a) R= fur-2-yl; R$_1$= Ph	(g) **R= 4-ClPhOCH$_2$;**
(b) R= fur-2-yl; R$_1$= 4-FPh	**R$_1$= 4-MePh**
(c) R= cyclopropyl; R$_1$= Ph	(h) **R= 4-FPh; R$_1$= 4-ClPh**
(d) R= 4-FPh; R$_1$= Ph	(i) **R= 4-FPhCH$_2$; R$_1$= MePh**
(e) R= H; R$_1$= 4-ClPh	(j) **R= 4-BrPhCH$_2$; R$_1$= 4-ClPh**
(f) R= 2-OMePh; R$_1$= 2-ClPh	
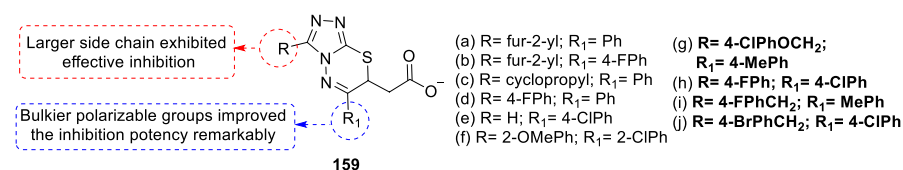
Fig. 71 SAR and uridine 5′-diphosphate-galactopyranose mutase (UGM) inhibitory potential of 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine derivatives

5 Pharmacokinetic Profile

Pharmacokinetics is the study of processes by which drugs are absorbed, distributed, metabolized and eliminated from the body. Performing standard experimental procedures for absorption, distribution, metabolism and excretion (ADME) profiling for a vast number of compounds would have been time-consuming and economically unfeasible. Most drug molecules fail to enter the market due to their adverse pharmacokinetic properties. Recently, computer simulation platforms are gradually becoming popular as they can accelerate the pharmacokinetic development of the drug molecule [124, 125]. In the primary phase of drug discovery, in silico approaches for the prediction of pharmacokinetics of drug candidates play an essential role in terms of cost effectiveness, time factors and avoidance of the ethical problems that arise from a large number of animals required for experiments. A successful lead should obey Lipinski's rule of five, which states that the drug should be easily absorbed, distributed to its target site, not easily metabolized, and excreted easily.

The drug-likeness of a compound was assessed according to Jorgensen's rule of three [126] and Lipinski's rule of five [127], which indicates that a chemical substance can be orally active in humans by estimating structural or physicochemical properties (descriptors) which are important for drug pharmacokinetics. Jorgensen's rule of three states that Caco-2 cell permeability (P_{Caco}) > 22 nm/s, predicted aqueous solubility ($P\log S$) > − 5.7 and number of primary metabolites < 7. Lipinski's rule of five states that orally active drug has no more than one violation of the following criteria: molecular weight < 500 Da; hydrogen bond donors ≤ 5; hydrogen bond acceptors ≤ 10; and octanol/water partition coefficient (log P) ≤ 5 (or $M\log P$ not greater than 4.15) [128, 129]. Topological polar surface area (TPSA) ≤ 140 Å^2 and the number of rotatable bonds ≤ 10 are also considered additional criteria used to determine oral bioavailability [130].

Triazolo[3,4-*b*][1,3,4]thiadiazine derivatives have been identified as compounds with broad-spectrum activity, high safety, and excellent pharmacokinetic properties in a variety of studies. Therefore, analysis of arrangement of substituents around triazolothiadiazine scaffold (Fig. 73) might provide useful information to construct novel triazolothiadiazine derivatives with better biological proficiency.

Çevik et al. [76] calculated ADME parameters of benzimidazole-triazolothiadiazine derivatives **105** by QikProp 4.8 software for comparing with those of 95% of known drugs. All compounds **105** were found in accordance with the Jorgensen's rule of three and Lipinski's rule of five for drug-likeness properties by causing no more than one violation. According to predictions of ADME properties for compounds **105e** and **105l**, including molecular weight 433.489 and 467.934, respectively, the number of rotatable bond values (1), only one hydrogen bond donor group, hydrogen bond acceptors 6 and log$P$ 4.468 and 4.96, respectively, it can be suggested that the active compounds **105e** and **105l** have desirable pharmacokinetic properties.

Sathyanarayana et al. [113] reported that novel [1,2,4]triazolo[3,4-*b*][1,3,4] thiadiazine derivatives **146** bearing benzimidazole moiety obeyed most of the
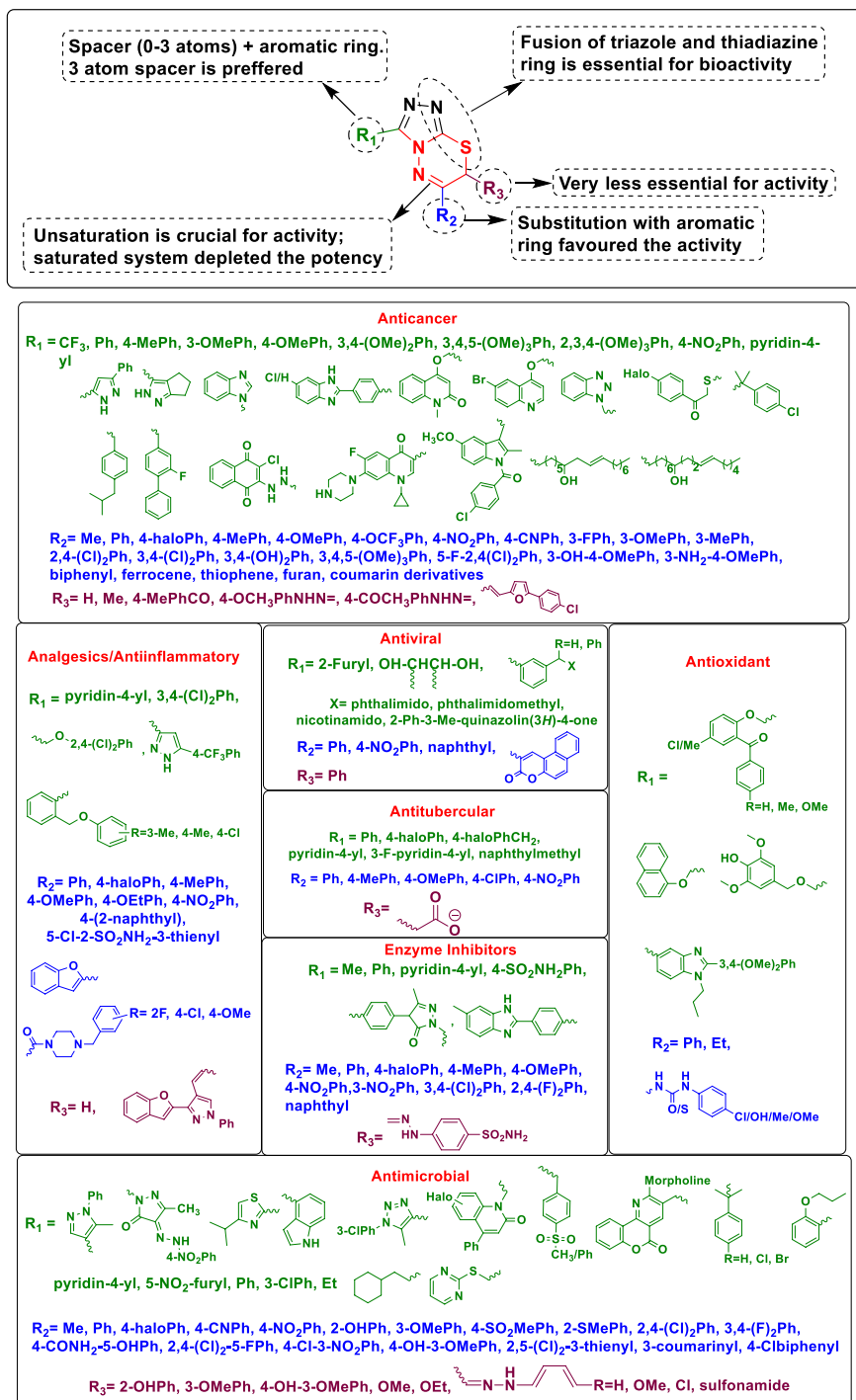
Fig. 72 Structural modifications on triazolothiadiazine scaffold and their effect on bioactivities
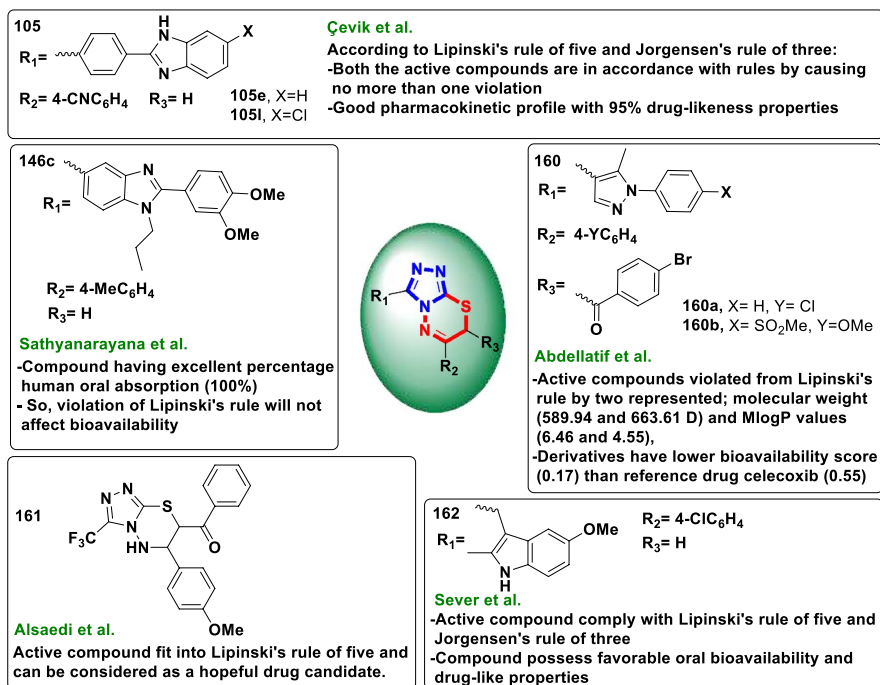
Fig. 73 Structural variations on triazolothiadiazine moiety and their predicted pharmacokinetic profile

ADME parameters. Most of these compounds showed TPSA < 80 $\mathring{A}^2$, indicating their good intestinal absorption. The most potent antioxidant **146c** showed excellent percentage human oral absorption (100%). Instead of having molecular weight ≥ 500 Da, these compounds have high percentage of human oral absorption and a violation of Lipinski's rule of five will not affect oral absorption [131]. QikProp predicted the number of possible metabolic reactions of these compounds in the range 2–5 (recommended range 1–8), indicating the compounds can easily reach the target site. Moreover, the LogKp values for compounds **146** range from -1.35 to -3.26 (acceptable range -8.0 to -10.0), which indicates that compounds **146** can be administrated dermally.

In silico ADME properties of the most selective triazolothiadiazine derivatives **160a** and **160b** as a new oral anti-inflammatory agent were accessed using the SwissADME web tool by Abdellatif et al. [132], which revealed that both compounds violated Lipinski's rule of five by two represented by molecular weight (589.94 and 663.61 Da, respectively) and MlogP values (6.46 and 4.55, respectively). Consequently, these triazolothiadiazine derivatives have lower bioavailability scores (0.17) compared with that of the reference drug celecoxib (0.55).

Alsaedi et al. [133] synthesized triazolo[3,4-*b*][1,3,4]thiadiazine derivative **161** as potent VEGFR2 inhibitor with anti-hepatocellular carcinoma activity and predicted pharmacokinetic properties using SwissADME tool. Compound **161** showed good gastrointestinal (GI) absorption, high oral bioavailability, and fitted well in

the Lipiniski's rule. ADME prediction revealed that compound **161** has a desirable pharmacokinetic profile and can be considered as a hopeful drug candidate.

Sever et al. [134] predicted pharmacokinetic profiles of triazolothiadiazine derivatives using QikProp program of Schrodinger's Maestro molecular modelling package. Compounds exhibited human oral absorption percentages ranging from 91.010% to 100.000%. All compounds comply with Lipinski's rule of five and Jorgensen's rule of three. On the basis of these findings, they are expected to possess favorable oral bioavailability and drug-like properties. According to in silico ADME studies for triazolothiadiazine **162**, the total solvent accessible surface area (SASA) was 622.356 Å^2, the predicted octanol/water partition coefficient (logPo/w) was 5.224, logarithm of predicted binding constant to human serum albumin, logK_{HSA} value was 0.957 and the predicted apparent Caco-2 cell permeability P_{Caco-2} was 1763.718. These results were in agreement with recommended range, thus indicating compound **162** to possess a favorable ADME profile.

6 Molecular Modeling

In receptor-based drug design, the 3D structure of the binding site is modelled on the target with the aid of molecular simulations. Docking experiments are carried out to identify the binding sites through computationally model ligand–receptor interactions as well as lead candidates from the binding energy scores [135]. A literature survey reflected the prominence of the 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine structural motif in the drug designing aimed at diverse pharmacological activities by its robust interactions with various receptors. Extensive research has focused on structural attributes of 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines responsible for its bioactivity on particular targets via computational studies (docking and molecular dynamics). Structural variations on triazolothiadiazine scaffold and their interactions with different biological receptors are shown in Fig. 74. 1,2,4-Triazolo[3,4-*b*][1,3,4]thiadiazine derivative **111j** exhibited promising anticancer potential, which is supported by several interactions of its core nucleus with amino acids Lys[352], Ala[250], Lys[254], Leu[248], Asn[258] and the H-bond between oxygen atom of its trimethoxyphenyl ring and the thiol group of Cys[241] in the colchicine binding site of tubulin polymerase [82]. The methylpropylphenyl moiety of triazolothiadiazines **88b–d** inserted into a hydrophobic basin formed by Val[349], Leu[352], Phe[518] and Ile[523] amino acids of the COX-1 protein to form a hydrophobic interaction [59]. In compound **113h**, the N2 of the triazole ring and NH at position-3 interacted with Val[135] via two H-bonds inside the ATP binding pocket of GSK-3β, one of the targets of anticancer activity of this compound [84]. Compound **104a** displayed H-bond interactions with Asp[479] and Lys[456] present at ATP catalytic binding domain of Topo-Iiβ [75]. Docking studies displayed that compound **102a** formed strong H-bond interactions with Ser[93], His[321] and His[154] in active site of tissue-nonspecific alkaline phosphatase (*h*-TNAP), while compound **102d** formed important interactions with the active site of intestinal alkaline phosphatase (*h*-IAP), which included two H-bonds between two nitrogen atoms of triazole ring and Arg[166] and metal interaction of carbonyl group of coumarin core with Zn^{2+} [73]. Docking studies indicated that compound **105e** could
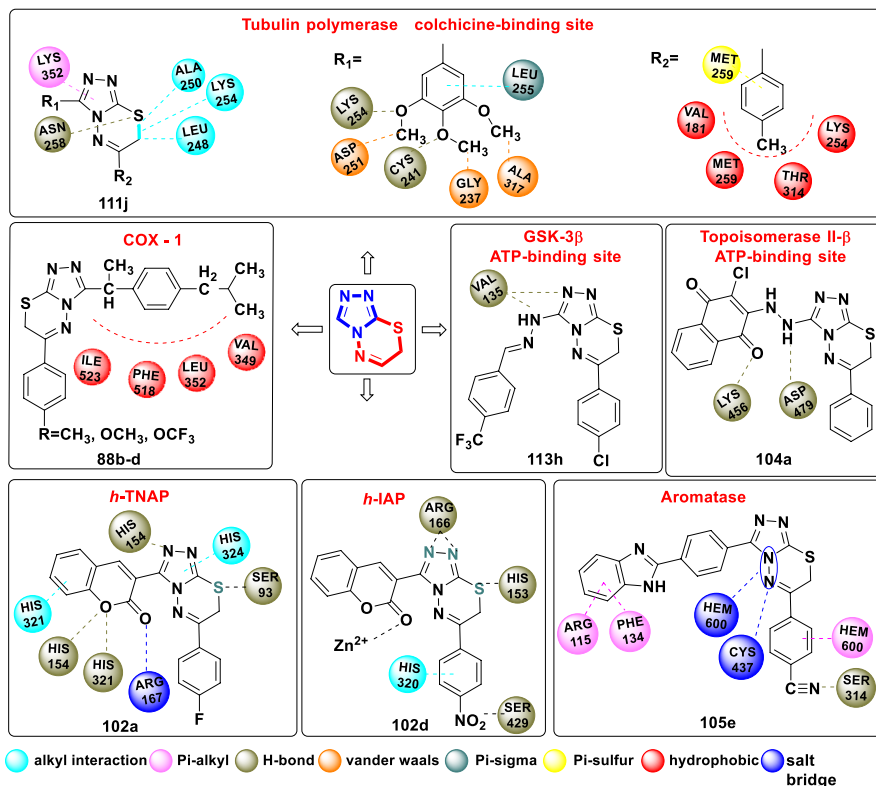
Fig. 74 Effect of structural modifications of triazolo[3,4-*b*][1,3,4]thiadiazine on molecular docking simulations with various receptors

inhibit aromatase enzyme through an H-bond interaction of CN group at the para-position of the phenyl ring with Ser^{314}. Besides, benzimidazole ring formed two π–π interactions with Arg^{115} and Phe^{134} and the triazolothiadiazine ring could interact by a salt bridge with the Hem^{600} molecule and Cys^{437} [76].

Further modifications and changes in substitution patterns at the C-3 and C-6 positions could result in higher affinity towards different receptors with additional interactions. This wide statistics will impart directions to researchers in innovating target selective, optimized and diverse 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine analogs for the treatment of multifactorial diseases.

7 Conclusion

In conclusion, the present review article deliberated the various synthetic protocols engaged for synthesis of diverse array of triazolothiadiazine analogs via multicomponent reactions (MCRs), conventional heating and visible-light-mediated

organic reactions. Moreover, the triazolothiadiazine scaffold is an important structural motif in biologically active compounds, exhibiting more potency than standard marketed drugs. This review presented an in-depth analysis of medicinal aspects of 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines for different biological activities such as anticancer, antimicrobial, analgesic and anti-inflammatory, antioxidant, antiviral, enzyme inhibitors and antitubercular activities. The activity profile of this scaffold particularly offers structural modifications at the C3 and C6 positions of the triazolothiadiazine ring to achieve new analogues. This study also revealed the structural diversity, biological importance and SAR examination of heterocyclic compounds containing a triazolothiadiazine nucleus, which will help the practitioners in further exploration of biological efficacy.

Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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