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REVIEW

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Insights into the medicinal chemistry of heterocycles integrated with a pyrazolo[1,5-*a*]pyrimidine scaffold

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Pyrazolo[1,5-*a*]pyrimidines are the dominant motif of many drugs; for instance, zaleplon and indiplon are sedative agents and ocinaplon was identified as an anxiolytic agent. The importance of this class of compounds lies in its varied and significant biological activities, and accordingly, considerable methods have been devised to prepare these compounds. Hence, other derivatives of this class of compounds were prepared by substitution reactions with different nucleophiles exploiting the activity of groups linked to the ring carbon and nitrogen atoms. The methods used vary through the condensation reactions of the aminopyrazoles with 1,2-allenic, enaminonitriles, enaminones, 1,3-diketones, unsaturated nitriles, or unsaturated ketones. Alternatively, these compounds are prepared through the reactions of acyclic reagents, as these methods were recently developed efficiently with high yields. The current review highlighted the recent progress of the therapeutic potential of pyrazolo[1,5-*a*]pyrimidines as antimicrobial, anticancer, antianxiety, anti-proliferative, analgesic, and antioxidant agents, carboxylesterase, translocator protein and PDE10A inhibitors, and selective kinase inhibitors.

1. Introduction

Heterocyclic pyrazolopyrimidines are a class of [5–6] bicyclic systems with three or four nitrogen atoms resulting in four mainly possible structural isomers. Alternatively, we highlight herein the biological importance and sample synthetic approaches of pyrazolo[1,5-*a*]pyrimidines; this class of compounds revealed varied and privileged biological significance. Hence, drugs containing a pyrazolo[1,5-*a*]pyrimidine skeleton obtained synthetically are widely applied; for instance, indiplon (**1**), loredipIon (**2**), dorsomorphin (**3**), zaleplon (**4**), dinaciclib (**5**), ocinaplon (**6**), pyrazophos (**7**), and anagliptin (**8**) (Fig. 1),[1] which were used for the treatment of anemia, blocked arteries, and musculoskeletal disorders, possess a great affinity for translocator protein, and are used as sedative and anxiolytic drugs, *etc.*[2]

Scientists' interests in recent research are attentive to exploring the diverse and noteworthy biological characteristics of

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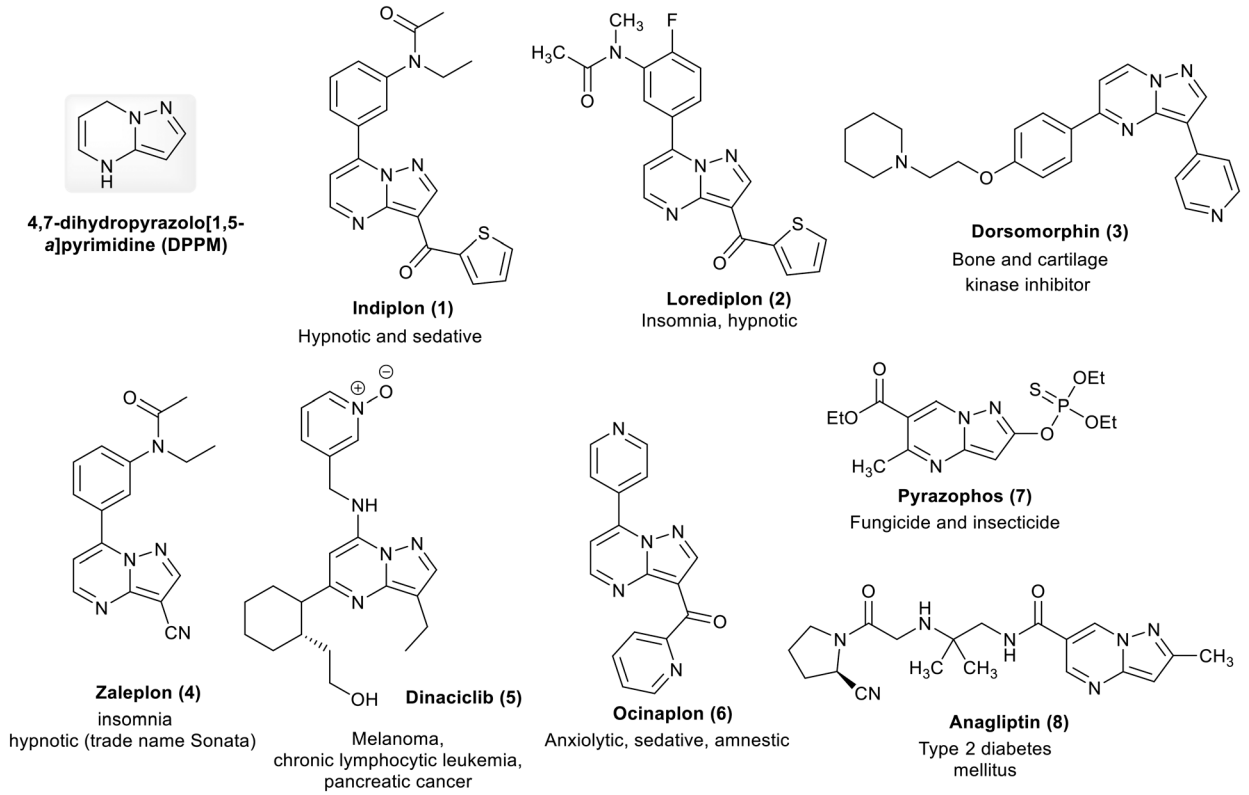

Fig. 1 Synthetic drugs incorporated with a pyrazolopyrimidine core.

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this class of compounds. These heterocyclic compounds have a prodigious impact that stemmed from their potential biological potency as anti-inflammatory, sedative–hypnotic, antitumor, antidiabetic, and anti-fever agents.[3–5] Moreover, these compounds provided potential inhibition for calcium channels in the human body,[6] besides their activities as antitumor, antimicrobial, antitrichomonal, antischistosomal, anticancer, antitumor, antitrichomonal, anti-inflammatory, antischistosomal, antidepressant, analgesic, antipyretic, and antiviral activities, in addition to sleep disorder treatment.[7–9] Besides, pyrazolo[1,5-*a*]pyrimidines also revealed privileged biological profiles that were extended by other researchers who reported their antitumor,[10,11] antimycobacterial,[12] antitrypanosomal, and antineuroinflammation activities.[13] Specifically, pyrazolo[1,5-*a*]pyrimidines demonstrated potent effects as anxiolytic,[14,15] antischistosomal, xanthine oxidase inhibitor,[16] antimicrobial,[9*b*,16] tuberculostatic,[17] cytotoxic,[18,19] antiproliferative,[20] antileukemic, and antianxiety agents,[21,22] along with their activities as inhibitors against KDR kinase,[23,24] AMP phosphodiesterase,[23] COX-2, and estrogen receptor ligands.[25]

Additionally, these compounds are also reported as selective inhibitors for Pim-1, HIV-1, CK2,[26] HCV polymerase, and c-Src kinase and COX-2-selective for acute ischemic stroke treatment.[27–33] Substituted pyrazolopyrimidines were reported as estrogen receptors, potential competitive antagonists,[34] and peripheral benzodiazepine receptors[35,36] and are applied in cancer therapy.[37] Furthermore, pyrazolo[1,5-*a*]pyrimidines were studied as CRF,[38] serotonin 5-HT,[39] GABA/GABAA,[4,40] and estrogen[41] receptor antagonists, as hepatitis C virus[42] and COX-2[33] inhibitors, and as potassium channel openers.[43] Also, pyrazolo[1,5-*a*]pyrimidines were applied as PET tumor detection agents,[44] for drug design,[45] and as dyes in photographic technology.[46] Pyrazolopyrimidines were also used to investigate cyanide recognition in water[47] and are known as selective inhibitors for DDR1.[48]

On the other hand, the chemistry of bicyclic systems and their use in pharmaceutical and medicinal fields attracted researchers' interest in recent years.[49–58] A comprehensive study of the synthetic routes adopted to prepare pyrazolo[1,5-*a*]pyrimidines has recently been published including all the synthetic courses from aminopyrazoles with different reagents or acyclic reactants under basic or acidic or mild conditions.[59] Also, other reviews discussed the protocols concerned for the synthesis of pyrazolo[1,5-*a*]pyrimidines with a non-comprehensive study.[60–62] The anticancer potency and protein kinase inhibitors were reviewed for pyrazolo[1,5-*a*]pyrimidines by Ismail *et al.*[63] Accordingly, the current review aimed to discuss the constructive and privileged biological potency of a heterocycle-integrated pyrazolo[1,5-*a*]pyrimidine hybrid that was published in recent years.

2. Model synthetic strategies

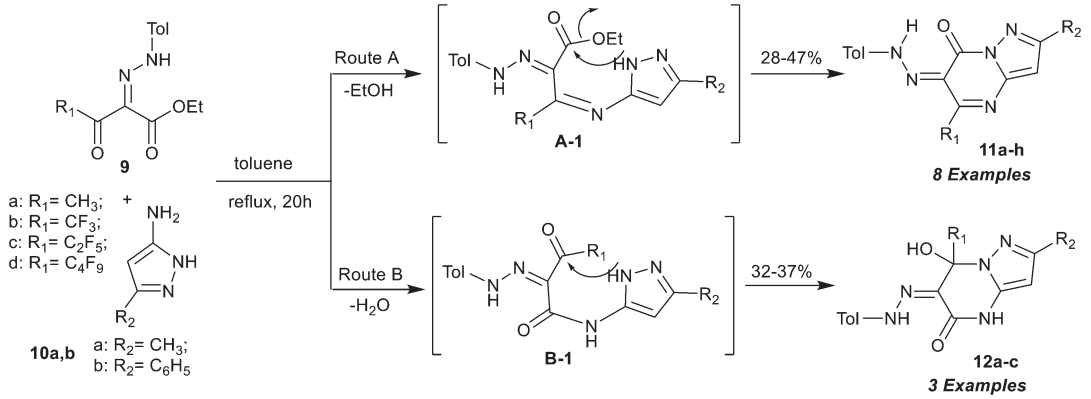
Recently, many techniques have been developed to prepare these heterocycles by exploiting the reactivity of aminopyrazole compounds towards numerous active precursors by condensation with 1,2-allenic ketones, enaminonitriles, enaminones, 1,3-dicarbonyl compounds, unsaturated ketones, and many other reagents. Also, many methods have been developed under different catalytic conditions by changing the solvent type (acidic, basic, and neutral medium) or through other eco-friendly protocols or using acyclic reactants. The importance of each method used to synthesize these molecules lies in the ease of obtaining products from readily available reactants with high efficiency, yields, purity, and faster reaction rate.[59] In particular, pyrazolopyrimidines could be efficiently prepared by condensation reactions of aminopyrazoles with 1,2-allenic ketones,[64] enaminonitriles, enaminones,[39*b*,65,66] 1,3-dicarbonyl, α,β-unsaturated carbonyl compounds, or ethoxymethylenes,[33,67] β-halovinyl aldehydes under regioselective catalytic conditions,[68] or 1,3,5-trisubstituted pentane-1,5-diones through C–C bond cleavage with a loss of one carbonyl group[69] under either microwave or ultrasonic irradiation conditions.[70,71] The compounds of this series might be obtained by three-component reactions[72] or through two-step processes by condensation of aroyl acetonitriles with hydrazine hydrate[73] or sulphonyl hydrazides,[74] deprived of the isolation of the aminopyrazole compounds. The tremendous biological profile of this class of compounds inspires the researchers to synthesize other molecules with a pyrazolo[1,5-*a*]pyrimidine nucleus.

2.1. Bicyclic ring construction from β-diketones

Burgart *et al.*[75] recently reported the synthesis of two sequences of 6-(2-tolylhydrazono)-dihydropyrazolopyrimidinones **11** and **12** by reactions of β-diketones **9** with aminopyrazoles **10** in toluene at reflux temperature. In this sequence, two possible routes A-1 and B-1 are postulated, in which the exocyclic amino group of aminopyrazoles has two prospects for condensation with carbonyl ketone (route A) or nucleophilic attack at the carbonyl ester (route B) in absence of acid medium that may activate the endocyclic imino group (NH) of the pyrazole ring. Compounds of series **11** have two *E*- and *Z*-isomers, in which the *Z*-isomers are more stable due to the possible six-membered ring construction by hydrogen bond formation of NH-hydrazo with the carbonyl oxygen at the pyrimidine ring (Scheme 1).

Kamal *et al.*[76] conveyed the reactions of 1*H*-pyrazole **10b** with β-diketones **13** under catalytic conditions by applying CAN at room temperature to yield the corresponding methyl carboxylates **14**. The respective carboxylic acids **15** were attained by basic hydrolysis of the methyl carboxylates **14** (Scheme 2).

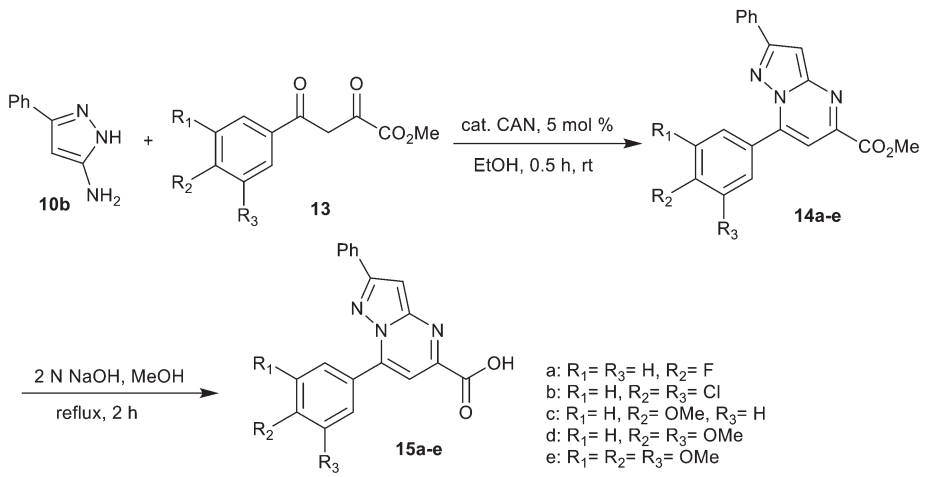
Guerrini *et al.*[77] reported the synthesis of two series of pyrazolopyrimidinones **19** and **20** by one-step reactions of aminopyrazoles **16** with β-diketones **17** or **18** under solvent-free conditions in diglyme (Scheme 3). Selleri *et al.*[78] previously reported the synthesis of **19a**, **19e**, and **19j** of the first series (R_1 = Ph).



11a: R_1= CH_3, R_2= CH_3; b: R_1= CH_3, R_2= C_6H_5; c: R_1= CF_3, R_2= CH_3; d: R_1= CF_3, R_2= C_6H_5; e: R_1= C_2F_5, R_2= CH_3; f: R_1= C_2F_5, R_2= C_6H_5; g: R_1= C_4F_9, R_2= CH_3; h: R_1= C_4F_9, R_2= C_6H_5

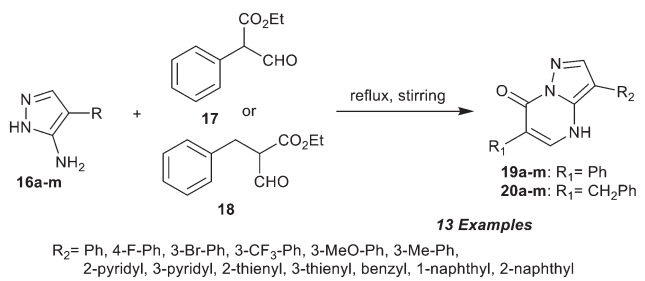
12a: R_1= C_2F_5, R_2= C_6H_5; b: R_1= C_4F_9, R_2= CH_3; c: R_1= C_4F_9, R_2= C_6H_5

Scheme 1 Synthesis of 6-(2-tolyl-hydrazono)-dihydropyrazolo[1,5-*a*]pyrimidinones.



Scheme 2 Synthesis of pyrazolopyrimidine-5-carboxylic acids.

Tran *et al.*[79] reported the synthesis of bicyclic pyrazolopyrimidines **23** by reactions of the corresponding aminopyrazoles **21** with β-diketones, specifically, acetylacetone (**22a:** R_2 = Me), and 3,5-heptanedione (**22b:** R_2 = Et) in ethanol at reflux temperature through cyclocondensation progressions. The cleavage of the isopropyl group was accomplished by reduction with $AlCl_3$ in CH_2Cl_2 at room temperature to yield the respective phenol analogs **24** (Scheme 4).

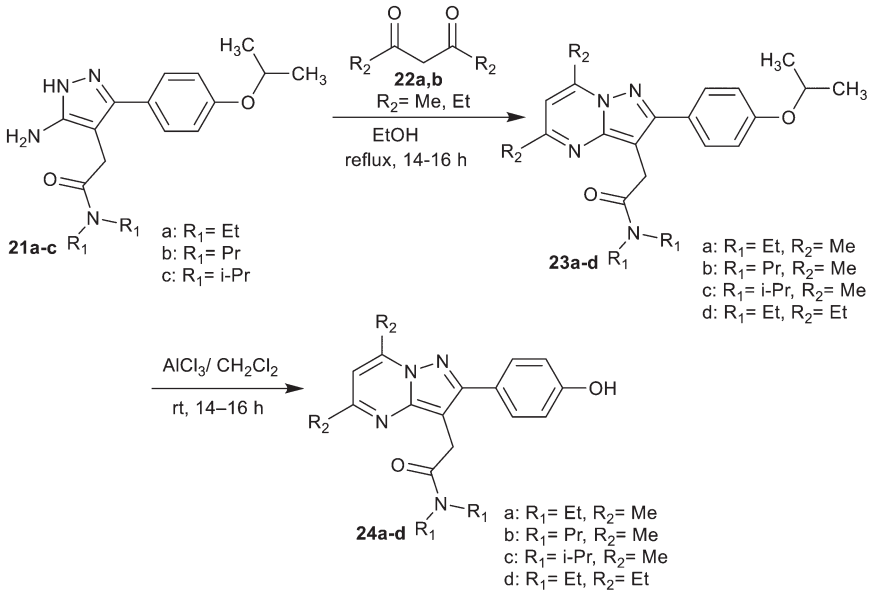


Scheme 3 Synthesis of 3,6-disubstituted pyrazolopyrimidinones.

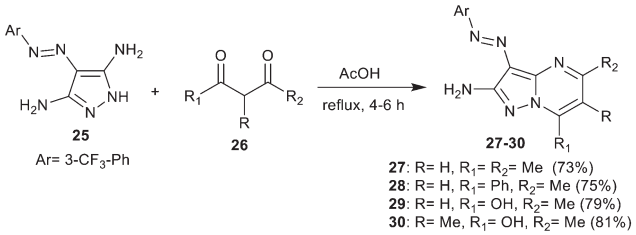
The aminopyrazole **25** straightforwardly reacted with β-diketones **26** in acetic acid at reflux temperature to provide the desired bicyclic pyrazolopyrimidines **27–30** in 73–81% yield (Scheme 5). The cyclization step is commonly dependent on the structural characteristics of the diketone and the acid medium, which enable the tautomerization of the amidine system and hence allow the nucleophilic attack of the endocyclic imino group that is more reactive before the nucleophilic attack of the exocyclic amino group at the carbonyl group of the diketone.[80]

2.2. Bicyclic ring construction from α,β-unsaturated ketones

The bicyclic systems pyrazolopyrimidine **33** were proficiently synthesized in 74% yield through the reactions of aminopyrazole **31** with 3-aryl-1-phenylprop-2-en-1-one **32** in ethanol containing piperidine at reflux temperature (Scheme 6), as reported by Fayed *et al.*[80] The basic medium enables the condensation of the carbonyl group of the unsaturated ketone with the exocyclic amino group at the first step. In hand, the endocyclic imino group attacks the unsaturated carbon followed



Scheme 4 Synthesis of substituted pyrazolopyrimidines.

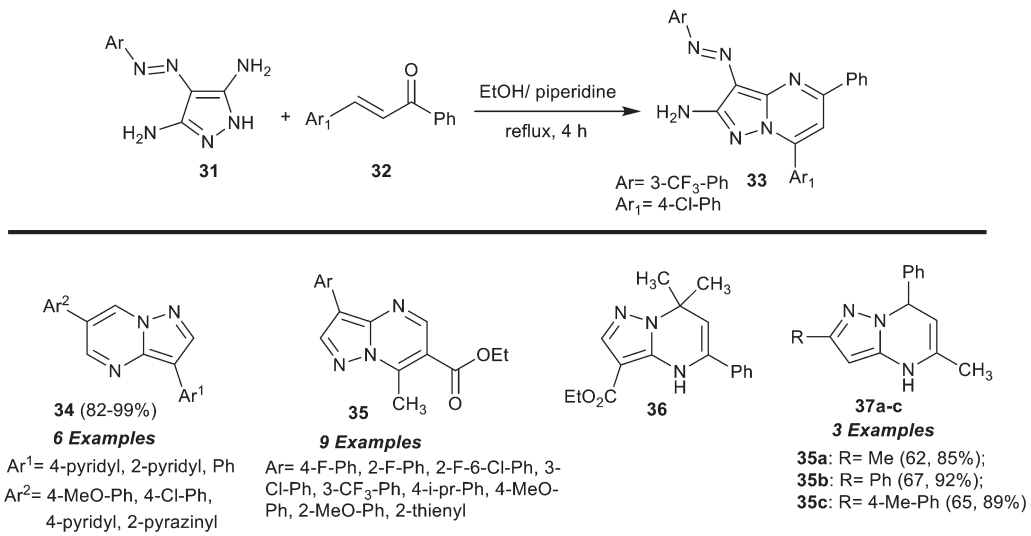


Scheme 5 Synthesis of bicyclic systems from β-diketones.

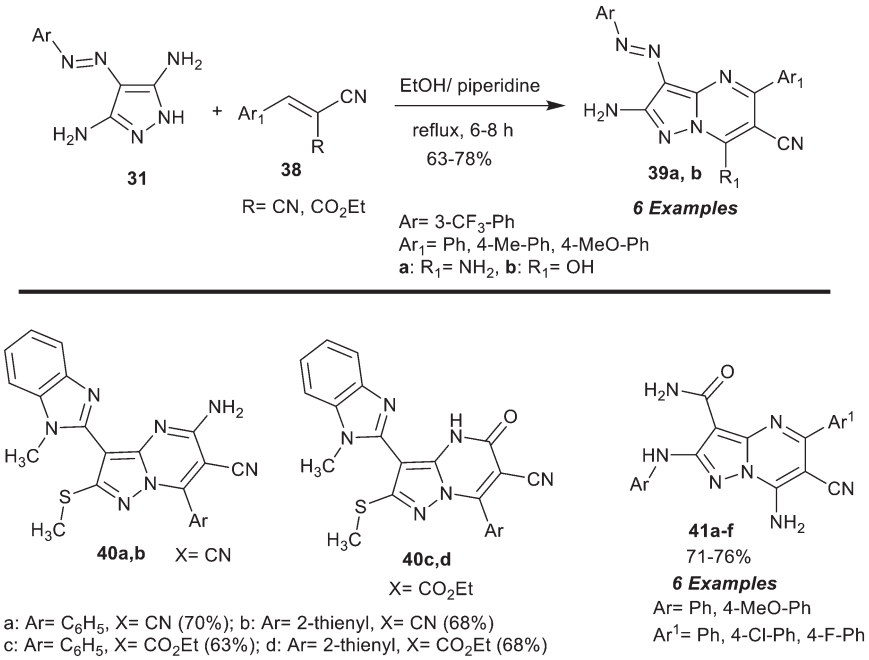
by cyclization and auto-oxidation steps. Also, a series of pyrazolopyrimidines **34** were proficiently synthesized in 40–60% yield by Daniels *et al.*[70c] from reactions of 4-aryl-1*H*-pyrazol-5-amines with 3-hydroxy-2-arylacrylaldehydes under microwave irradiation conditions. In addition, the ethyl carboxylates **35** were obtained with reduced yields (≈35%) from reactions of 4-aryl-1*H*-pyrazol-5-amines with unsaturated ketones in methanol under reflux conditions.[45] The reactions of ethyl 5-amino-1*H*-pyrazole-4-carboxylate with 3-methyl-1-phenylbut-2-en-1-one in DMF/NaH gave the ethyl carboxylates **36.**[81] Under conventional or ultrasonic conditions, cyclocondensation of 5-aminopyrazoles with benzylidene acetone in *n*-butanol gave 4,7-dihydropyrazolo-pyrimidines **37a–c.**[82]

2.3. Bicyclic ring construction from α,β-unsaturated nitriles

Unsaturated nitriles are reactive synthons for the bicyclic ring construction of pyrazolopyrimidines by reactions with aminopyrazole. Hence, Fayed *et al.*[80] correspondingly defined



Scheme 6 Synthesis of bicyclic systems from unsaturated ketone.



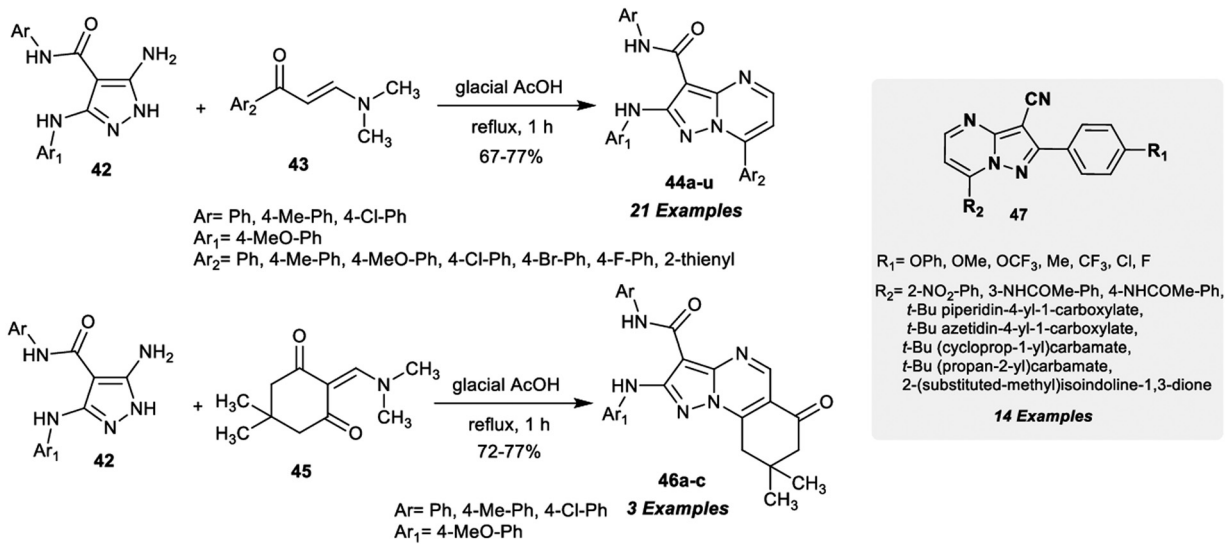
Scheme 7 Synthesis of bicyclic systems from unsaturated nitriles.

the synthetic routes of bicyclic pyrazolopyrimidines **39a** and **39b** in 63–78% yield by reactions of aminopyrazoles **31** with α,β-unsaturated nitriles **38** in ethanol/piperidine at reflux temperature (Scheme 7). Also, the analogous pyrazolopyrimidines **40a–d**[83] and carboxamides **41a–f**[84,85] were synthesized from the reactions of aminopyrazoles with α,β-unsaturated nitriles under different conditions, predominantly the utility of catalytic piperidine in boiling ethanol.

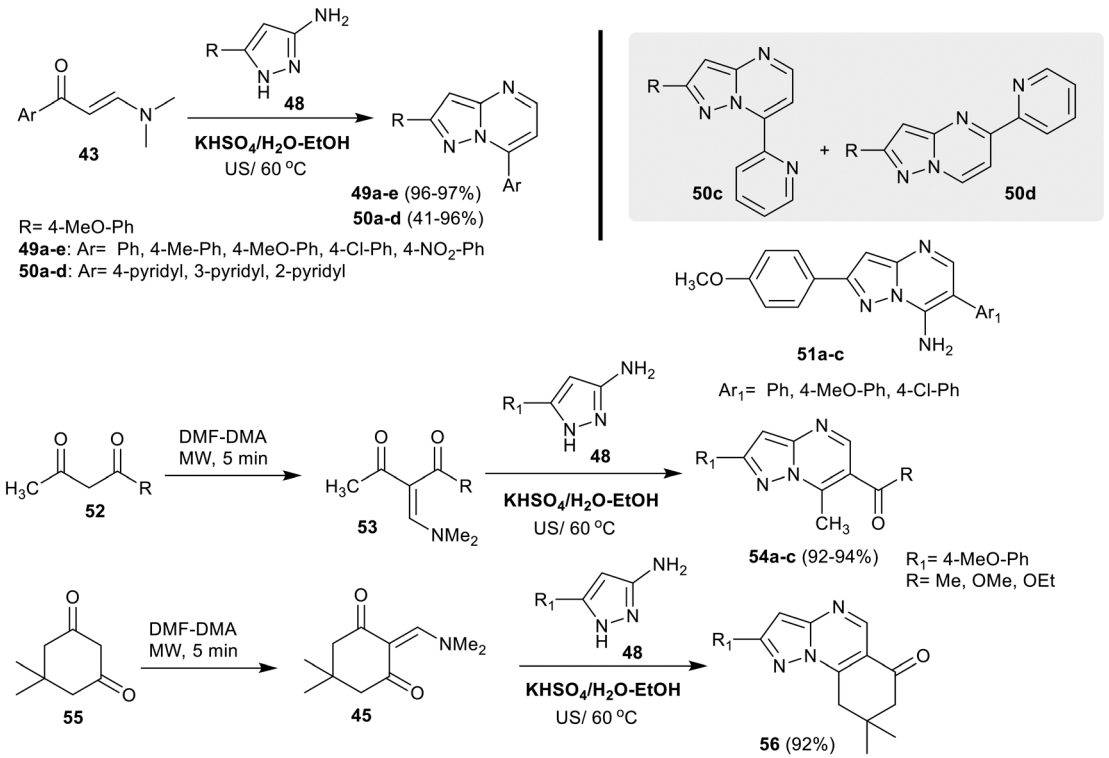
2.4. Bicyclic ring construction from enaminones

El-Naggar *et al.*[86] have synthesized two series of pyrazolo[1,5-*a*]pyrimidines **44** and **46** in moderate yields by reactions of aminopyrazoles **42** with enaminones **43** and **45**, respectively. The acid medium activates the endocyclic imino group of the pyrazole ring to start the first nucleophilic attack; hence the α-carbon of enamine is in a more electrophilic position. Awad[87] and Askar,[84] in similar way, reported analogous reactions for anticancer assessment. Guo *et al.*[88] reported the synthesis of pyrazolopyrimidines **47** by reactions of the respective aminopyrazoles with enaminones in refluxing acetic acid (Scheme 8).

Kaping's group[89–93] have recently specified the synthesis of pyrazolopyrimidine analogs from the reactions of aminopyrazoles with enaminones or enaminonitriles through



Scheme 8 Reactions of aminopyrazoles with enaminones.



Scheme 9 Synthesis of bicyclic pyrazolo[1,5-*a*]pyrimidines under ultrasonic irradiation conditions.

facile synthetic routes. Consequently, two series of bicyclic heterocycles **49** and **50** were greenly synthesized through the reactions of enaminones **43** with aminopyrazole **48** catalyzed by potassium bisulfate under ultrasonic irradiation conditions. The reaction of 5-aryl-1*H*-pyrazol-3-amine **48** with 3-(dimethylamino)-1-(pyridin-2-yl)prop-2-en-1-one yielded a mixture of regiospecific isomers **50c** and **50d**. The analogous bicyclic systems **51** were synthesized by reactions of the corresponding aminopyrazoles with enaminonitriles. Also, enaminones **53** and **45** reacted with 5-aryl-1*H*-pyrazol-3-amine **48** under identical conditions to yield the desired pyrazolopyrimidines **54** and **56** (Scheme 9).[91]

3. Reactivity

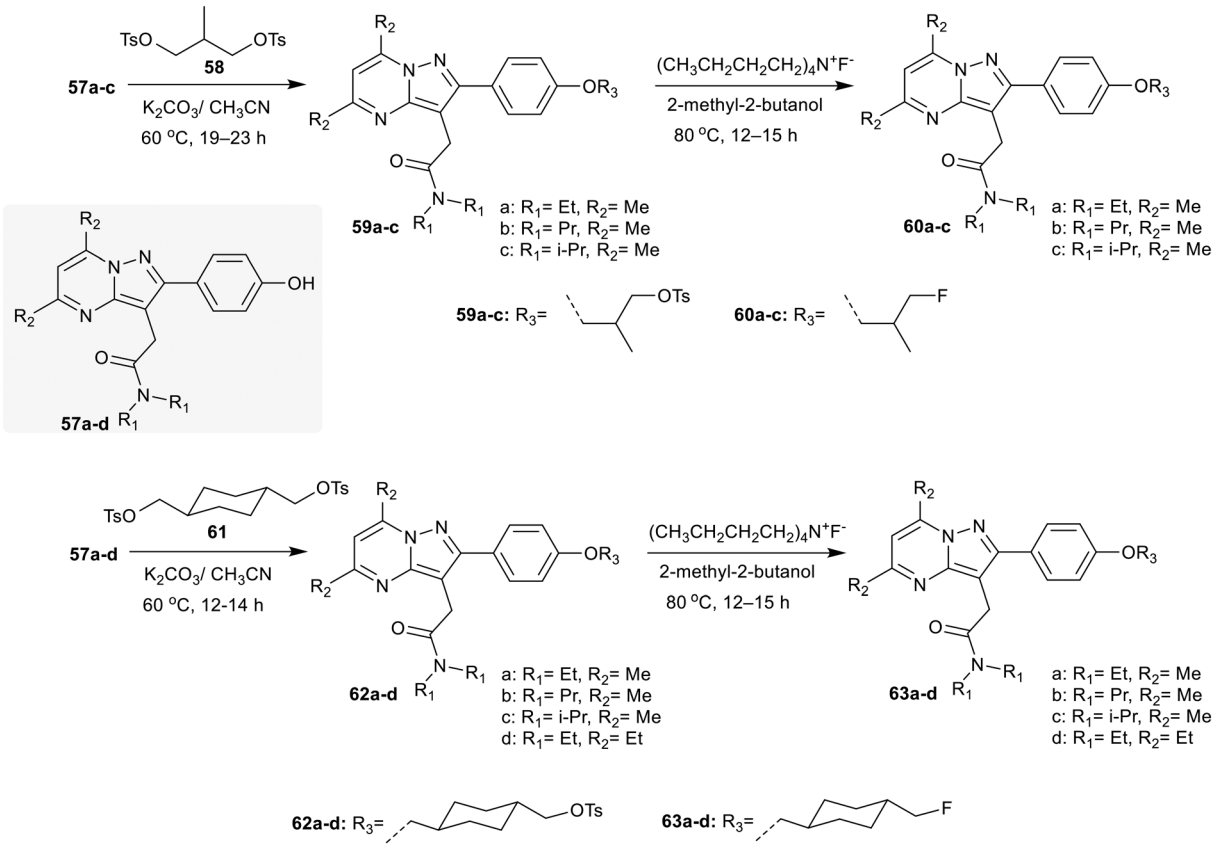
3.1. Reactivity of substituents linked to ring carbon

3.1.1. Synthesis of aryl ethers. The phenol derivatives **57a–d** readily reacted with ditosylates **58** and **61** in acetonitrile containing potassium carbonate to give the respective compounds **59a–c** and **62a–d** in 64–80% yield. Accordingly, these tosylate derivatives **59a–c** and **62a–d** reacted with *n*-Bu_4NF to yield the investigated fluorine-based **60a–c** and **63a–d** in 81–96% yields (Scheme 10).[79]

Kwon *et al.*[94] have detailed the synthesis of acetamides **67a–c** in excellent yields through an efficient procedure by reactions of aminopyrazoles **64** with β-diketones **65** in refluxing ethanol. The reductive bond cleavage of the isopropyl ethers **66a–c** was achieved by treatment with aluminum chloride in dichloromethane at room temperature, yielding the respective phenols **67a–c**. The alkylation of the phenol moiety of compound **67a–c** was accomplished by treatment with ditosylates in acetonitrile containing potassium carbonate to yield the alkylated ether products **68** and **70** with tosyl substituents. Subsequent fluorination of **68** and **70** with *n*-Bu_4NF yielded the particular fluoro-alkyl derivatives **69** and **71** (Scheme 11).

3.1.2. Synthesis of diamides. Palladium-catalyzed Buchwald–Hartwig coupling reactions of iminobenzophenone with aryl bromides **72** under heating conditions in the presence of a ligand, *i.e.* xanthphos yielded the respective imines **73**. Further acidic hydrolysis of the imines **73** with ethanol containing hydrochloric acid yielded the arylamines **74**. Next, the amide analogs **76** were synthesized by the reactions of acid chlorides **75** with the amines **74**. Base hydrolysis of the ester group of **76** was achieved using lithium hydroxide to give the acids **77**. Coupling of the acid derivatives **77** with various amines **78** yielded the diamides **79** using HATU as a coupling agent (Scheme 12).[95]

Predominantly, the reactions of *N*-alkyliodide salts with methylamine in an alcoholic solution did not give the recyclization products. Therefore, the reaction of **80a** with methylamine **81** yielded *N*-methyl-3-phenyl-1*H*-pyrazol-5-amine (**82**) as a major product, besides the formation of aminopyrazole **48** and the dealkylation product **83a**. On the other hand, compound **80b** reacted with methylamine to afford the dealkylated product **83b** as a major product besides aminopyrazole **48**, methylaminopyrazole **82**, and the acyclic molecule **84**. Structure **84** did not cyclize due to the



Scheme 10 Synthesis of fluorine-based pyrazolopyrimidinyl-*N*,*N*-disubstituted-acetamides.

low nucleophilicity of the pyrazole ring that hindered the cyclization step (Scheme 13).[96]

3.1.3. Synthesis of unsaturated ketones. Catalytic reduction of the esters **85** with DIBAL-H in dichloromethane yielded the desired aldehydes **86.** Condensation of the aldehydes **86** with acetophenones **87** catalyzed by barium hydroxide in methanol at room temperature gave the anticipated chalcone-based pyrazolopyrimidines **88** (71–91%). Accordingly, the Weinreb–Nahm reactions of aldehydes **86** with *N*,*O*-dimethylhydroxylamine hydrochloride catalyzed by trimethylaluminum in dry dichloromethane yielded the respective amides **89**. Grignard reaction of **89** with methyl magnesium bromide in dry tetrahydrofuran gave the ketones **90.** Further condensation of **90** with various aldehydes **91** in the presence of barium hydroxide in methanol at room temperature produced the respective chalcone-based pyrazolopyrimidines **92** (79–92%%) (Scheme 14).[97]

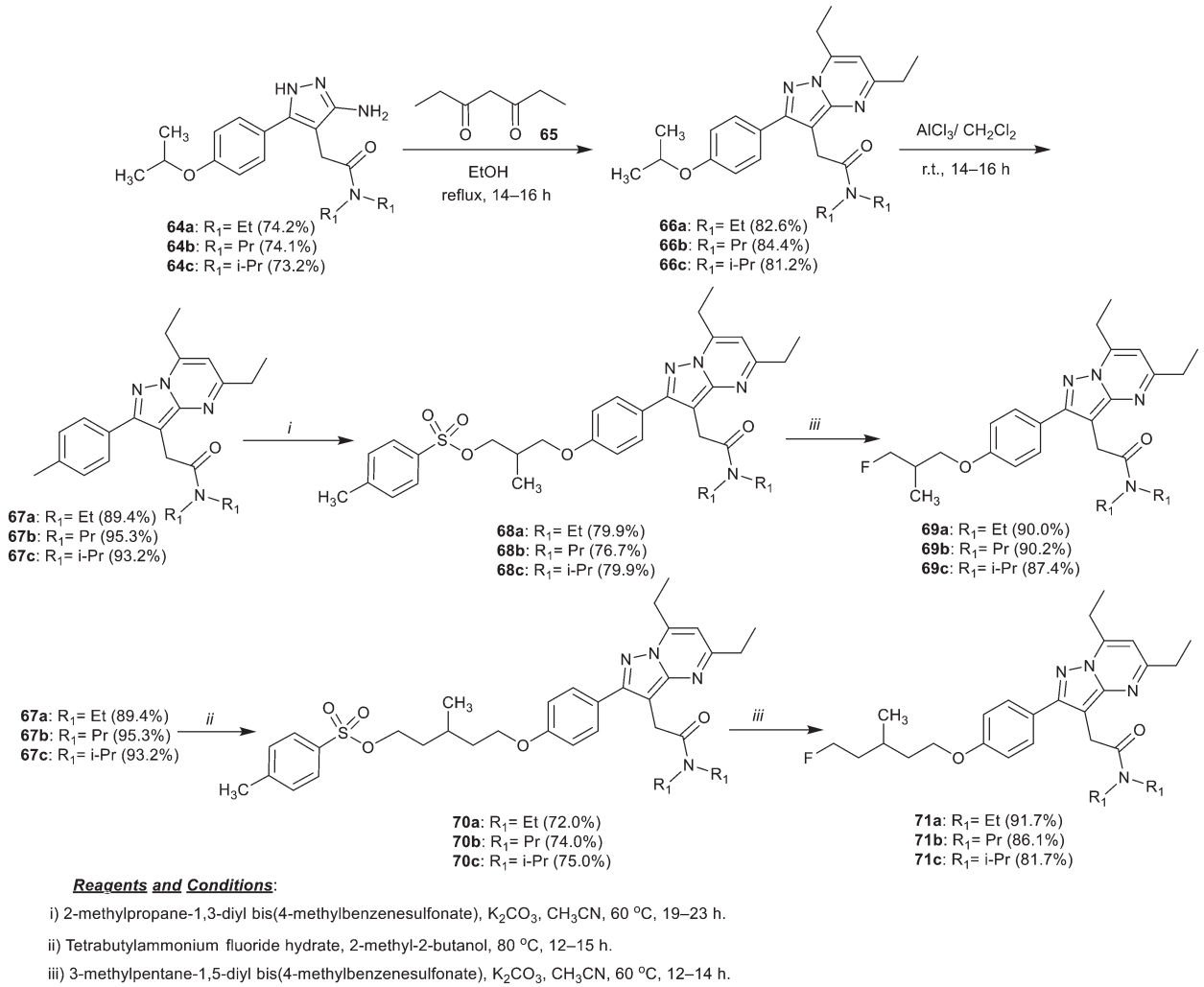
3.1.4. CH-arylation reactions. Palladium-catalyzed microwave-assisted one-pot reactions of ethyl acetate **93** with aryl halides in toluene accompanied by hydrolysis of the ethyl ester group with lithium hydroxide under microwave irradiation conditions yielded a series of pyrazolopyrimidines **94a–k** in 49–80% yields (Scheme 15). The aryl halide, *i.e.* 1-bromo-3-methylbenzene, is preferred for the synthesis of the target compound **94e** (Ar = 2-Me-Ph) with the best yield (64%) over 1-chloro-3-methylbenzene or 1-iodo-3-methylbenzene. The sequence of these reactions involved a direct CH-arylation and subsequent saponification–decarboxylation step-reactions.[98] The procedure enables the arylation process with high yields, short reaction time, and reduced steps than the outdated methods that involved chlorination of the hydroxy substituents and reaction with (benzyl)zinc(II) chlorides.[8c,99]

3.2. Reactions involving substituents attached to ring nitrogen atoms

The *N*-alkyliodide salts **96a–c** were obtained by reactions of pyrazolopyrimidines **95a** and **b** with alkyl halides such as methyl and ethyl iodides. The bridge nitrogen atom is not favored for alkylation since its lone pair of electrons was included in the aromatization of the ring system. The alkylation was achieved at N4 as indicated by spectral data (Scheme 16).[96]

3.3. Synthesis and reactivity of polycyclic systems

El-Essawy *et al.*[100] reported the reactivity of hydroxyl substituents at the pyrimidine ring. Therefore, the chlorination of hydroxy derivatives **97** and **100** with phosphorus oxychloride yielded the dichlorinated products **98** and **101**, respectively. Nucleophilic substitution reactions of compounds **98** and **101** with sodium azide yielded the bis-azido derivatives **99** and **102.** The reactions of chloro-substituted analog **101** with primary amines, *i.e.* phenyl hydrazine **104**, thiourea or aryl or alkyl



Scheme 11 Synthesis of pyrazolopyrimidinyl-*N*,*N*-alkyl-acetamides.

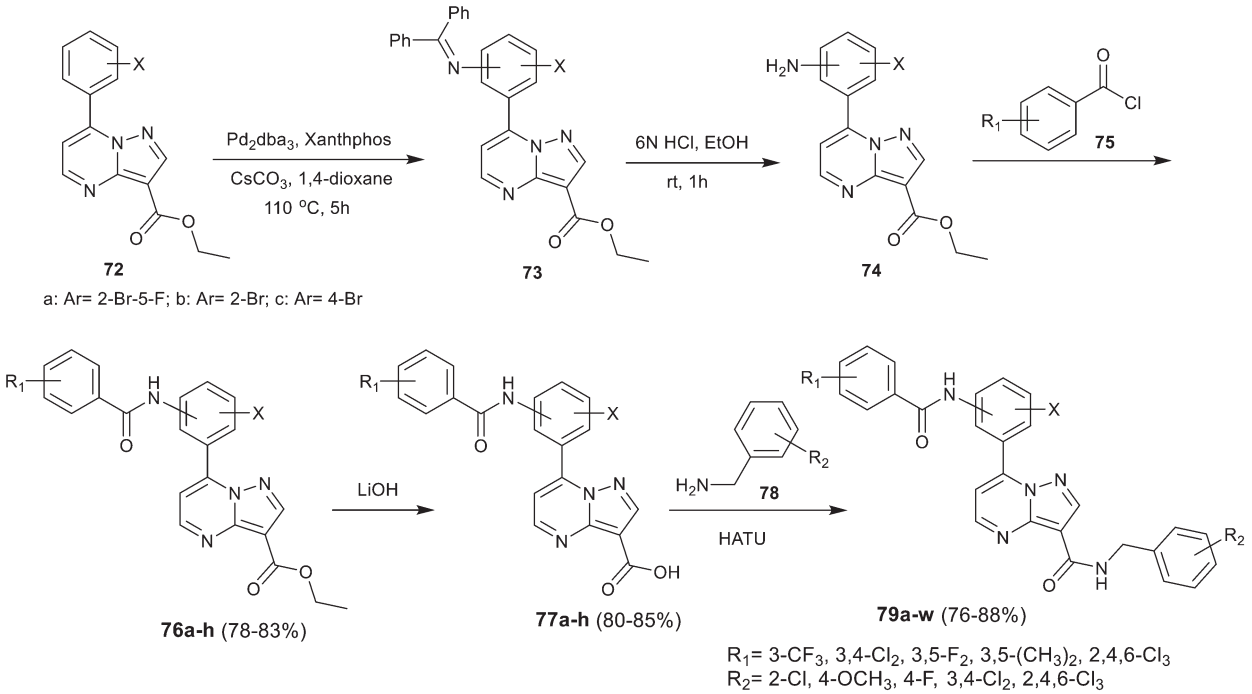
amines in ethanol or ethanol-containing base yielded the tetracyclic systems **103**, **105**, and **106** in 78–90% yield. The primary amines in these reactions enable the cyclization process through the substitution of both chlorine atoms (Scheme 17).

Attaby and Eldin[101] reported the synthesis of tricyclic pyrazolo–dihydropyrimido–pyrimidinone systems **112** through two-step reactions. Accordingly, the reactions of enaminonitriles **107** and **108** with formic acid yielded the isolated formimidic acids **111** in 60–78% yield through the tautomerization of intermediates **110**. The carboxamides **109** also generated intermediates **110** by heating with anhydrous formic acid. The cyclization steps of compounds **111** were accomplished by heating in an acetic acid/acetic anhydride mixture to yield the anticipated products **112** through a cyclocondensation process (Scheme 18).

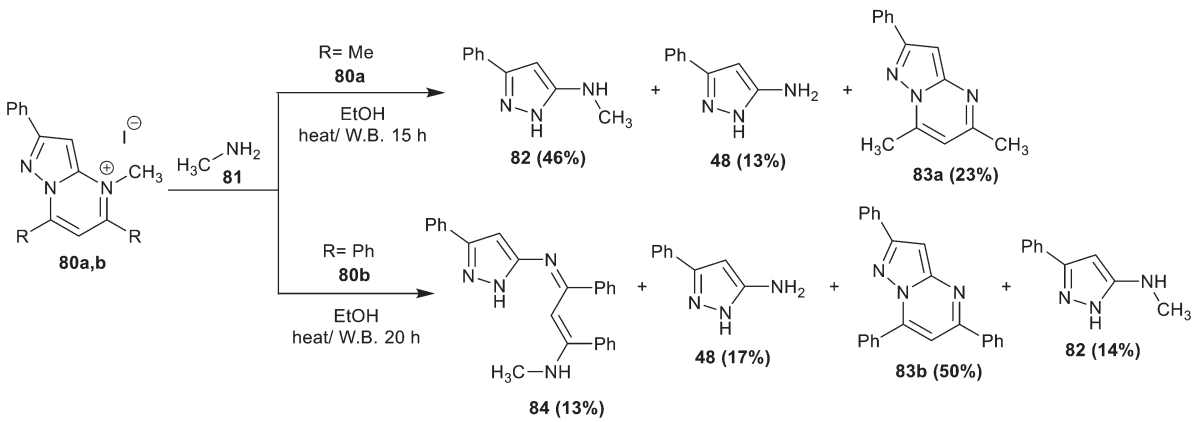
3.4. Synthesis of binary heterocycles

The reactivity of hydrazide **113** was examined in the reactions with each ethyl ethoxyacrylate **114** and arylidene malononitrile **116** in boiling ethanol to produce the respective cyclocondensation products **115** and **117**. The cyclization of the pyrazole ring was accomplished by nucleophilic attack of the amino group of hydrazide at the C3 position of compounds **114** and **116** with the elimination of the ethanol molecule followed by intramolecular nucleophilic attack of the imino group at the nitrile group to give products **115** and **117**. Treatment of cyclic aminoester 6 with triethyl orthoformate yielded the (ethoxymethylene)amino-1*H*-pyrazole **118** in an excellent yield. The reaction of compound **118** with aryl amines in acetonitrile yielded the respective bicyclic pyrazolo[3,4-*d*]pyrimidinones **119a** and **b**, with the formation of compound **115** as a by-product (Scheme 19).[102]

In a comparable route, condensation of hydrazide **120** with anhydrides in a choline chloride : urea system as a deep eutectic solvent (DES) (it behaves like ionic liquids), which has hydrogen bond acceptors and donors, yielded the 1*H*-pyrrole-2,5-diones **121a–d** in 85–92% yield. The mechanism involved the furan-2,5-dione ring-opening and cyclocondensation steps. Treatment of pyrazolo[1,5-*a*]pyrimidine **122** with 3-acetyldihydrofuran-2(3*H*)-one (**123**) in phosphorus oxychloride yielded the tricyclic **124** as a major product with the formation of the tetracyclic product



Scheme 12 Synthesis of diamide derivatives.



Scheme 13 Reactions of *N*-alkyliodide salts with methyl amine.

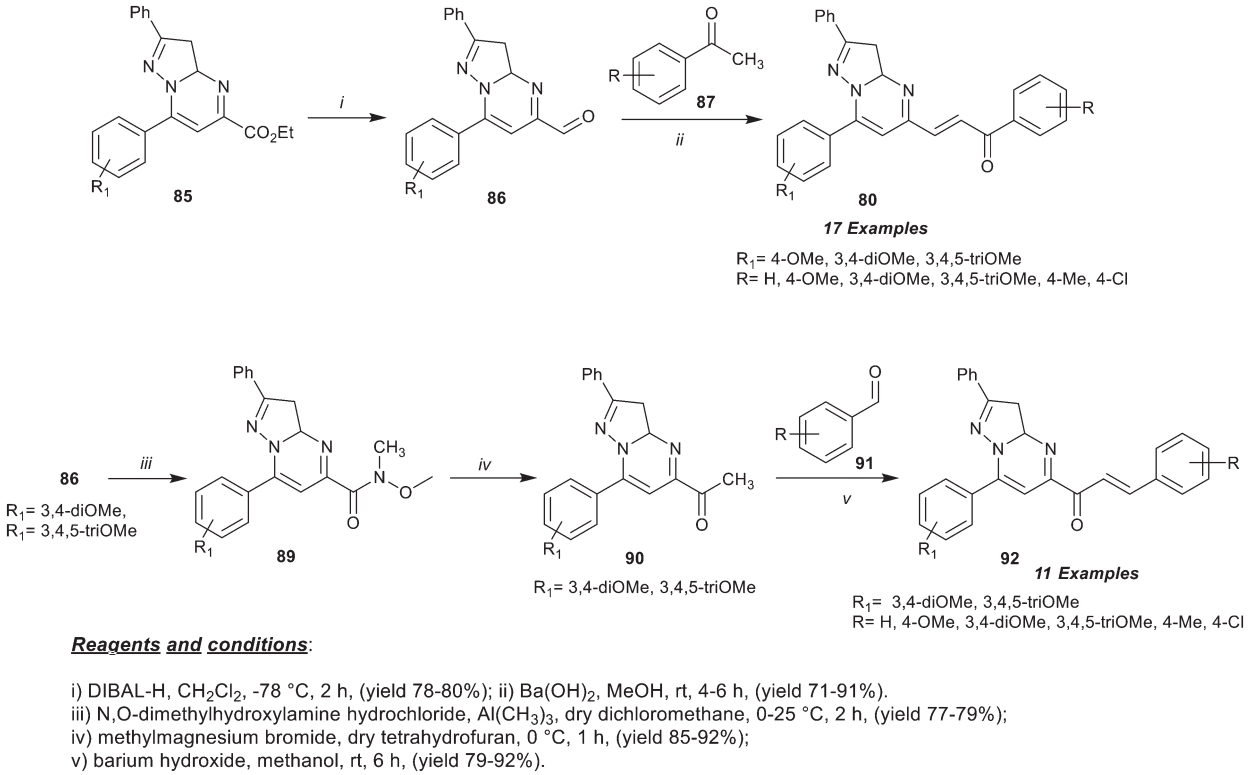
125 as a by-product (Scheme 20). The unexpected product **124** was formed through furan ring cleavage utilizing the reactivity of C6 of the pyrimidine ring, cyclization, and chlorination of the hydroxyl groups with phosphorus oxychloride.[102]

4. Biological characteristics

4.1. Antimicrobial activity

Previously, Novinson *et al.*[103] have identified the antifungal activities of a series of 7-alkyl-aminopyrazolopyrimidines. Recently, pyrazolopyrimidine **128** was synthesized with a worthy yield (83%) by condensation of **126** with enaminone **127** (Scheme 21). Compound **128** exhibited potent antifungal activity at a concentration of 50 μg mL^{-1} against *G. zeave*, *A. solani*, *P. asparagi*, and *C. arachidicola hori* with inhibition potency at 36.4, 45.1, 28.7, 39.6%, respectively. The herbicidal activity of compound **128** evaluated by a cup plate diffusion technique at 100 and 10 μg mL^{-1} indicated that the compound has a moderate activity at 65.8% and 40.3%, respectively, against *B. campestris*.[104]

Compounds **129a**, **129b**, **129c**, **130**, and **131** were tested as antimicrobial agents against Gram-positive bacterial species, *e.g. S. aureus* and *B. subtilis*, Gram-negative bacterial species, *e.g. P. aeruginosa* and *E. coli*, and fungal strains, *i.e. A. niger*, *A. flavus*, and *F. moniliforme*, using a disc diffusion assay for bacterial species and a dry weight method for fungal tests. Ampicillin and streptomycin were used as antibiotics for bacterial screening tests. The results demonstrated that compounds **129a–c** are the most potent antifungal agents compared to compounds **130** and **131** against all fungal



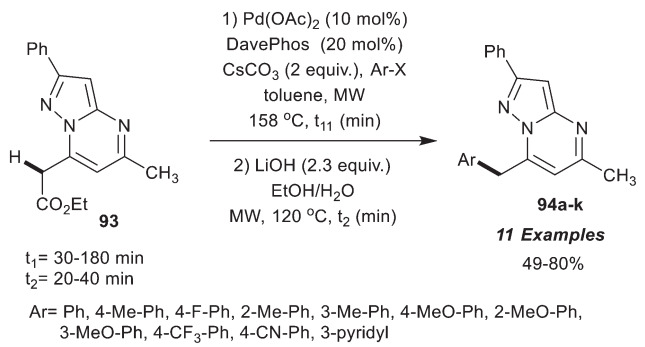
Reagents and conditions:

i) DIBAL-H, CH_2Cl_2, -78 °C, 2 h, (yield 78-80%); ii) $Ba(OH)_2$, MeOH, rt, 4-6 h, (yield 71-91%).
iii) N,O-dimethylhydroxylamine hydrochloride, $Al(CH_3)_3$, dry dichloromethane, 0-25 °C, 2 h, (yield 77-79%);
iv) methylmagnesium bromide, dry tetrahydrofuran, 0 °C, 1 h, (yield 85-92%);
v) barium hydroxide, methanol, rt, 6 h, (yield 79-92%).

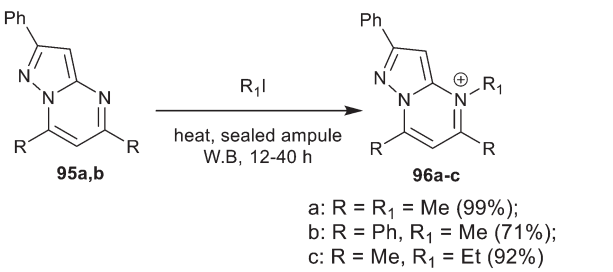
Scheme 14 Synthesis of chalcone-based pyrazolopyrimidines.

strains. Compound **129a** had the greatest potency against *S. aureus* with an inhibition zone at 9–12 mm, while compound **131** had the greatest potency against *E. coli* with an inhibition zone at 9–12 mm. The pyrazolopyrimidines are more superlative than pyrazolopyrimidin-5(4*H*)-one skeletons for potent antimicrobial results (Fig. 2).[83]

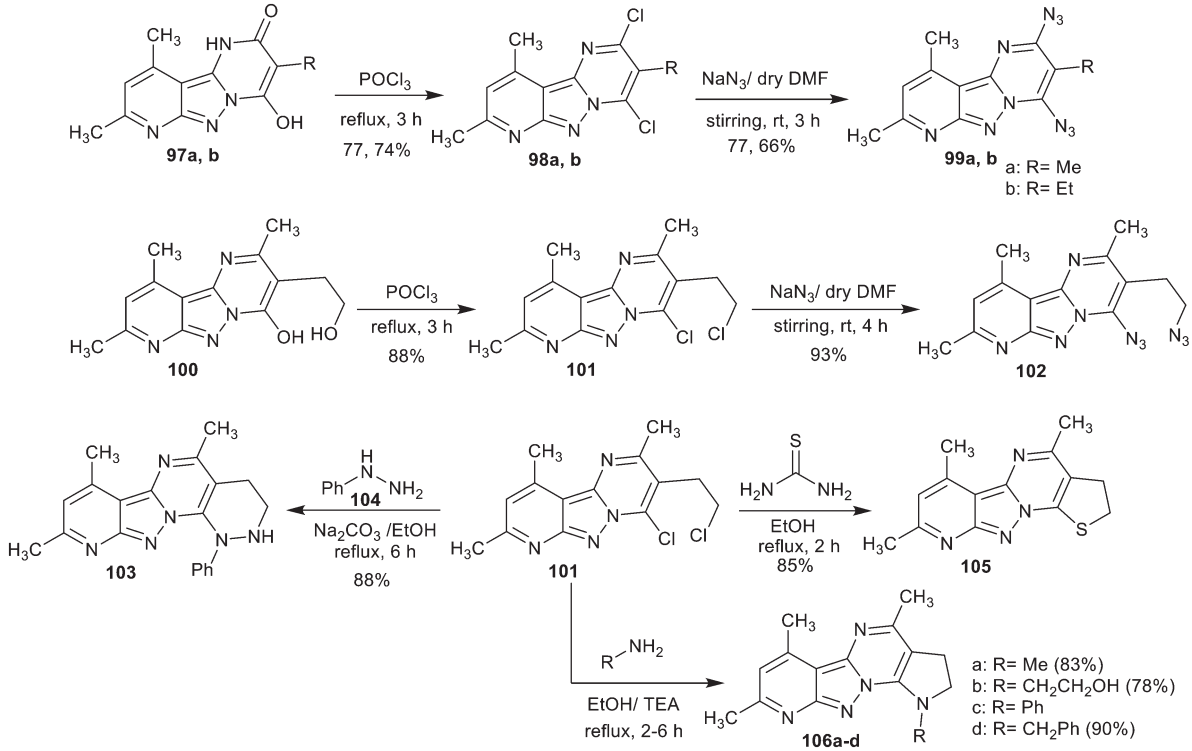
The antibacterial potency of pyrazolopyrimidines **115**, **117**, **119**, **121**, **124**, and **125** was assessed against *B. subtilis* and *E. coli* by an agar cup plate procedure. The compounds showed potent antibacterial activities against both bacterial strains compared to streptomycin (16.2 and 16.4 mm). The 1*H*-pyrazole **115** is more potent against both bacterial species (17.1 and 15.4 mm) than 1*H*-pyrazole **117** with inhibition zones at 16.1 and 14.2 mm, but compound **117** had a more effective MIC at 20 μg mL^{-1}. In addition, compound **119a** (14.3 and 13.1 mm) is more potent than compound **119b** (14.0 and 12.2 mm) against *B. subtilis* and *E. coli*. The order of the antibacterial potency of molecules of the series **121** was found as compound **121b** > **121d** > **121a** > **121c** against *B. subtilis*, whereas **121b** > **121c** > **121a** > **121d** against *E. coli*. Compound **124** (15.1 mm) is slightly more active than compound **125** (14.1 mm) against *B. subtilis*, while compound **125** (16.4 mm) is more active than compound **124** (15.3 mm) against *E. coli*. Compounds **121a** and **121d** have the highest MIC at 10 μg mL^{-1}; this is comparable to that of streptomycin (5 μg mL^{-1}). On the other hand, compounds **115**, **117**, **119**, **121**, **124**, and **125** were evaluated as antifungal agents against *C. albicans*, *C. tropicalis*, *A. niger*, and *A. clavatus*, and griseofulvin was used as a fungal standard. Compounds **117** and **121d** have the most potent antifungal agent *C. albicans* with an inhibition zone of 17.1 and 19.1 mm, higher than the result of griseofulvin (16.8 mm). Moreover, compound



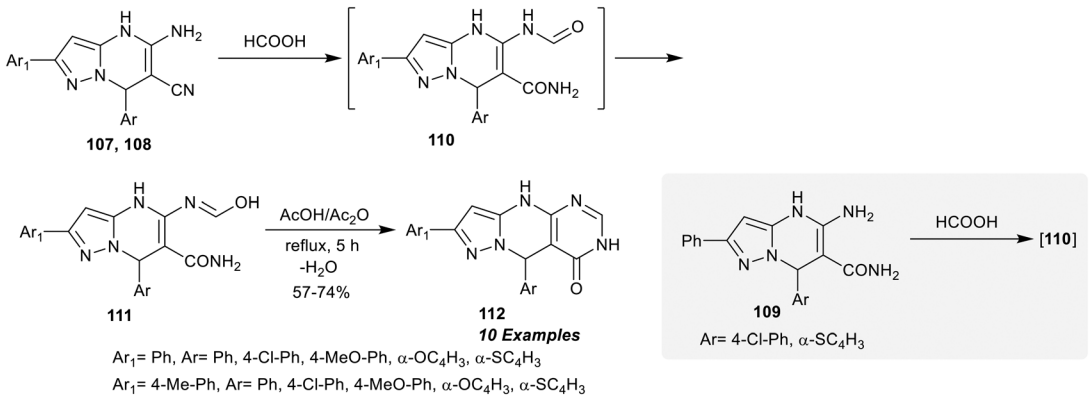
Scheme 15 Arylation of ethyl acetates.



Scheme 16 Synthesis of *N*-alkyliodide salts.



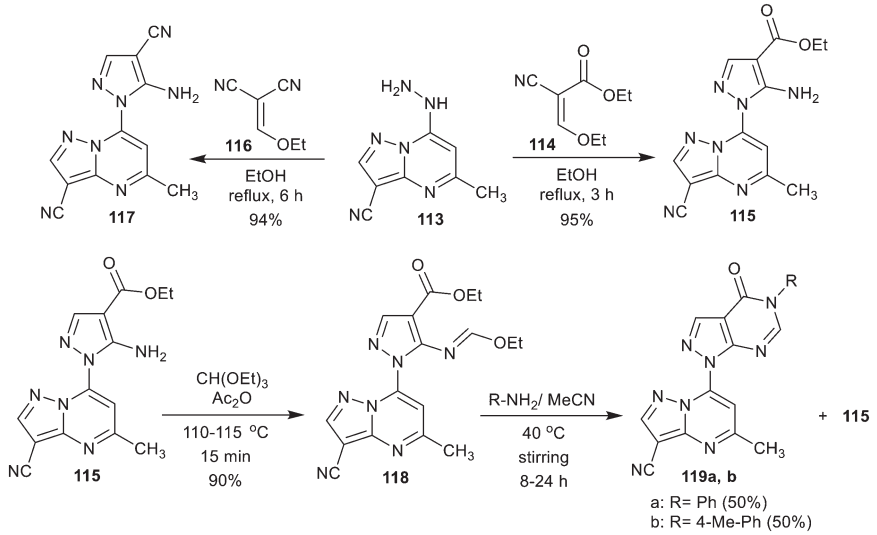
Scheme 17 Amination and synthesis of tetracyclic systems.



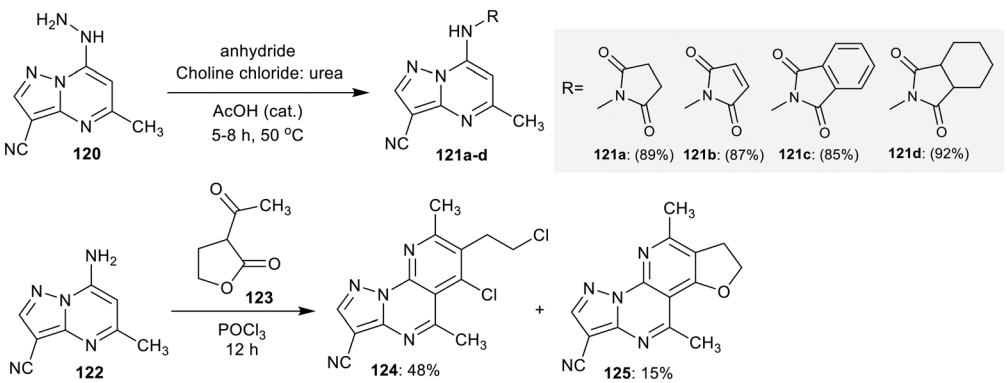
Scheme 18 Synthesis of tricyclic pyrazolopyrimidopyrimidinones.

121c had a slightly more potent activity (17.4 mm) than the antibiotic standard against *C. tropicalis*. Compounds **124** (17.2 mm) and **125** (17.7 mm) are the best active antifungal agents against *A. niger* and *A. clavatus*, respectively, with comparable potent activity to that of the antibiotic standard in both cases. In addition, the most effective compounds are **119b** and **121d** with MIC ranging from 10 to 15 μg mL^{-1}.[102] The SARs was intended as mentioned in Fig. 3.

Hassan *et al.*[84] have reported the synthesis of carboxamide analogs **132** by reactions of aminopyrazoles with enaminones, specifically, 3-(dimethylamino)-1-arylprop-2-en-1-ones (Ar_1 = Ph, 4-Me-Ph). In addition, the synthesis of bicyclic pyrazoloquinazoline-carboxamides **133** was accomplished by the reactions of aminopyrazoles with arylidene malononitriles (Ar_2 = Ph, 4-Cl-Ph, 4-F-Ph). Also, the reactivity of aminopyrazoles, *viz.*, 5-amino-3-(phenylamino)-1*H*-pyrazole-4-carboxamide and 5-amino-3-((4-methoxyphenyl)amino)-1*H*-pyrazole-4-carboxamide, was extended against the reactions with β-diketones, *i.e.* ethyl acetoacetate, acetylacetone, and 1,3-diphenylpropane-1,3-dione, through cyclocondensation reactions to yield carboxamide derivatives **134a** and **b** (Ar = Ph, 4-MeO-Ph) and **135a–d** (Ar = Ph, 4-MeO-Ph). The different cyclization processes for the construction of both series depended on the type of the β-diketones, in which the asymmetrical β-diketone, *i.e.* ethyl acetoacetate, gave the series of carboxamides **134a** and **b**, while the symmetrical β-diketones (X = Y = Me or X = Y = Ph) gave the carboxamides **135a–d**. The carboxamides **133a**, **133d**, **135a**, and **135c** were



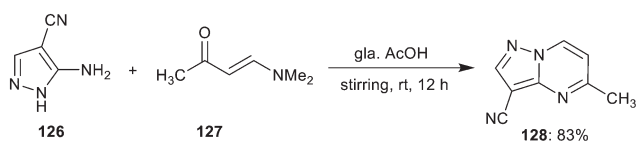
Scheme 19 Synthesis of binary 1*H*-pyrazoles and bicyclic pyrazolo[3,4-*d*]pyrimidinones.



Scheme 20 Reactions of amines with anhydrides and 3-acetyldihydro-furan-2(3*H*)-one.

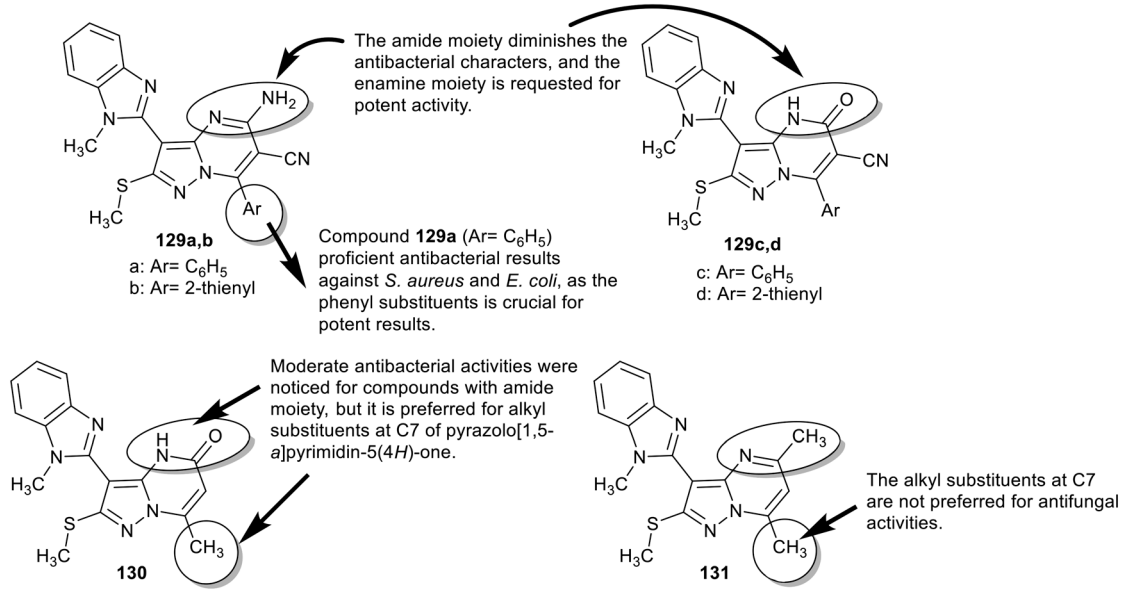
synthesized according to the method reported by Hafez[85] (Fig. 4).

Hassan *et al.*[84] have also evaluated the antimicrobial characteristics of carboxamides **132**, **133**, **134**, and **135** by a disc diffusion assay against *B. subtilis*, *S. aureus*, *E. coli*, and *P. aeruginosa* as Gram-positive and Gram-negative bacterial species using tetracycline as an antibiotic. Compounds **132a** and **135d** are the most potent analogs against all of the microorganisms with more potent activities than the antibiotic standard. The carboxamides of the series **133**, **134**, and **135** are inactive agents against the tested microbial species except for compound **135d**, which showed better activities against all the tested microbial strains. Compounds **132a–d** are active antibacterial agents with potent results (MIC: 3.9–7.81 μg mL^{-1} against *B. subtilis*, MIC: 7.81–15.62 μg mL^{-1} against *S. aureus*, MIC: 15.62–62.5 μg mL^{-1} against *E. coli*, and MIC: 7.81–62.5 μg mL^{-1} against *P. aeruginosa*). The antifungal activities for carboxamide derivatives **132**, **133**, **134**, and **135** were evaluated against *C. albicans*, *A. niger*, *A. fumigatus*, *P. chrysogenum*, and *F. oxysporum*. Amphotericin B was used as a standard antibiotic in this investigation of these series of carboxamides. It was found that compounds **133f** and **135b** are the most potent antifungal agents against *C. albicans* (MIC: 15.62 μg mL^{-1}); however, compound **132a** is the most potent against *A. niger* (MIC: 15.62 μg mL^{-1}). In addition, compound **133d** showed respectable antifungal activity against *A. fumigatus* with an inhibition zone at 14.0 ± 0.65 mm (MIC: 15.62 μg mL^{-1}). Compound **135d** exhibited the most potent antifungal activities against *P. chrysogenum* and *F. oxysporum* (MIC: 15.62 and 7.81 μg mL^{-1}) (Fig. 4).[84]



Scheme 21 Synthesis of pyrazolopyrimidine **128**.

Kaping *et al.*[92] have investigated the synthesis of compounds **136–139** by reactions of the corresponding 3-amino-*N*-phenyl-1*H*-pyrazole-4-carboxamide with enaminones under ultrasonic irradiation conditions. In addition, Kaping *et al.*[93] have also



Compounds	*Aspergillus niger*	*Aspergillus flavus*	*Fusarium moniliform*	*S. aureus*	*B. subtilis*	*P. aeruginosa*	*E. coli*
129a	0.51	0.36	0.19	++			+
129b	0.56	0.38	0.28				
129c	0.43	0.33	0.16		+		+
130	0.26	0.16	0.12	+		+	+
131	0.27	0.30	0.11	+		+	++

Fig. 2 SARs and antimicrobial results of the pyrazolopyrimidinones and pyrazolopyrimidines.

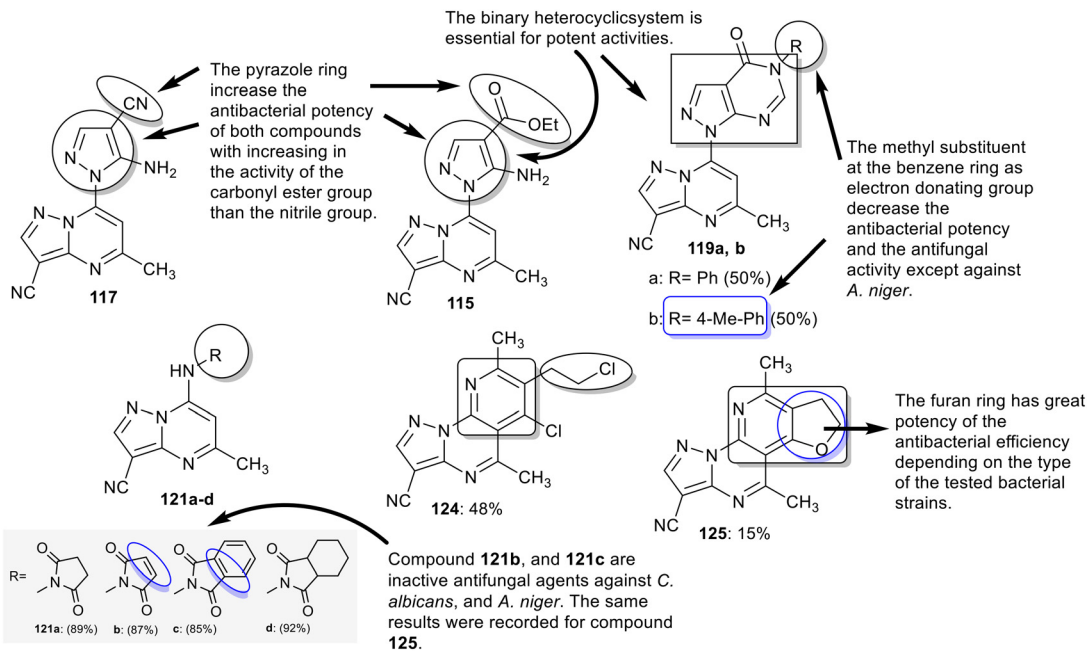
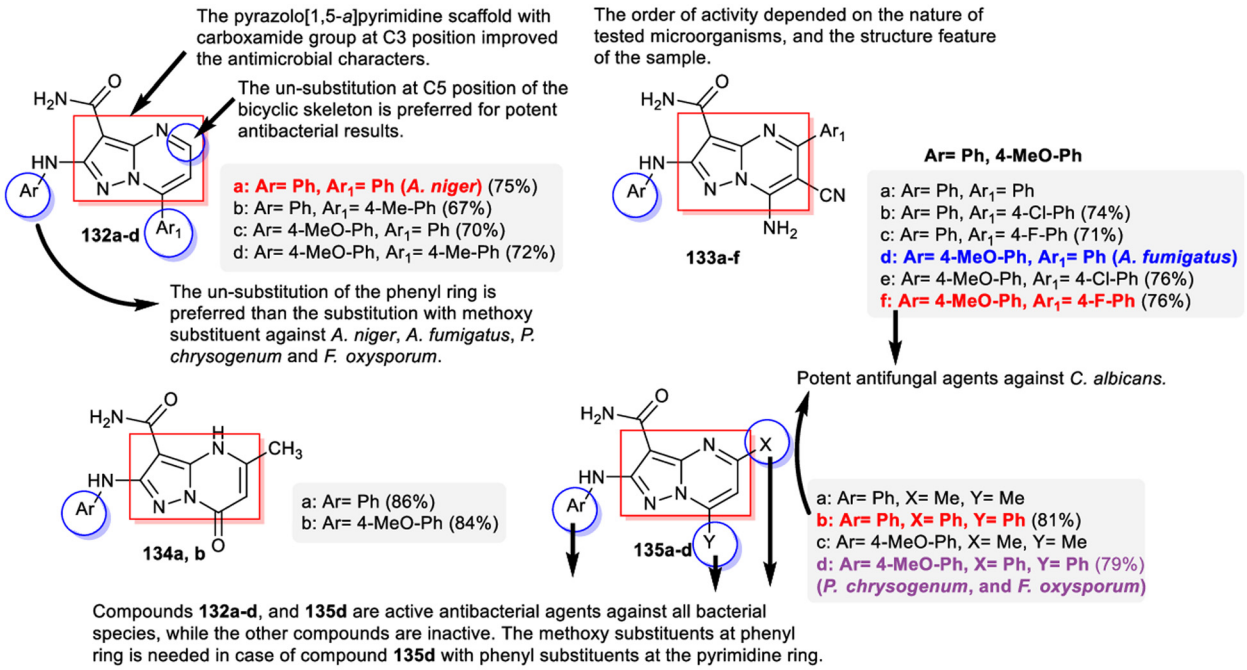


Fig. 3 SARs of potent bicyclic and binary pyrazolopyrimidines.

investigated the synthesis of analogous pyrazolopyrimidine-based carboxamidoantipyrine substituents by reactions of aminopyrazoles with enaminones under ultrasonic irradiation conditions. The antibacterial activity of 3-(carboxamido)-



Compounds	Inhibition zones in mm, (MIC in μg/mL)			
	B. subtilis	*S. aureus*	*E. coli*	*P. aeruginosa*
132a	12.0 ± 0.11 (3.90)	10.0 ± 0.25 (7.81)	7.0 ± 0.15 (15.62)	9.0 ± 0.54 (7.81)
135d	12.0 ± 0.33 (3.90)	9.0 ± 0.2 (7.81)	13.0 ± 0.22 (3.90)	10.0 ± 0.40 (15.62)
Tetracycline	25.0 ± 0.62 (31.25)	25.0 ± 0.51 (62.50)	23.0 ± 0.12 (15.62)	20.0 ± 0.05 (62.50)

Fig. 4 SARs of carboxamide analogs as potent antimicrobial agents.

pyrazolopyrimidines **136–139** was evaluated by Kaping *et al.*[92] against diverse bacterial species, *i.e. B. subtilis*, *E. coli*, *S. enterica*, and *S. aureus*. The results of the disc diffusion assay demonstrated that the compounds exhibited no activity against *B. subtilis*, and compounds **136a** (10 mm), **136d** (17 mm) and **139b** (19 mm) inhibited the growth of *S. aureus* with high potency relative to the result of ampicillin (21 mm). The compounds showed better antibacterial activities against both Gram-negative bacterial species. Compounds **136a** (10 mm), **136c** (10 mm), **136d** (10 mm), **136e** (11 mm), **138a** (12 mm), and **139c** (10 mm) revealed potent activities against *E. coli* compared to the antibiotic standard (11 mm). Also, compounds **138a** (23 mm), **138c** (11 mm), **138d** (20 mm), and **139a** (16 mm) showed potent antibacterial activities against *S. enterica* bacterial species relative to the result of ampicillin (23 mm). The SARs of the compounds as antibacterial agents are shown in Fig. 5.

Two series of pyrazolopyrimidines were prepared by reactions of aminopyrazoles with unsaturated ketones under reflux conditions as stated by Abdallah *et al.*[105] The compounds of both series were assessed as antimicrobial agents against Gram-positive bacteria, *i.e. B. subtilis*, *S. aureus*, Gram-negative bacteria, *i.e. E. coli* and *P. aeruginosa*, and fungal species, *i.e. A. flavus* and *C. albicans*, using a disc diffusion assay. The results revealed that compound **142b** has potent antibacterial activity against *B. subtilis* (12 mm) and *E. coli* (11 mm) relative to the results of the antibiotic ampicillin (26 and 25 mm). Definitely, compounds **142a**, **142b**, **142d**, **142h**, **144a**, **144e**, **144f**, **144h**, **144i**, **144l**, and **144n** revealed a good antibacterial spectrum against *B. subtilis* (9–14 mm). Compounds **144c**, **144g**, **144i**, and **144n** showed good activity against *S. aureus* (9–14 mm), while compounds **142b**, **144e**, **144i**, and **144n** revealed potent activities against *E. coli* species (9–12 mm). Consistently, compounds **142a**, **142b**, **142d**, **142h**, **144a**, **144e**, **144f**, **144i**, **144j**, **144l**, and **144n** presented potent antibacterial activities against *P. aeruginosa* species (9–12 mm). The other tests presented negative results, including the antifungal potency, as the compounds have no activity to inhibit or kill some bacterial or fungal species (Fig. 6).

Otherwise, pyrazolopyrimidines were obtained by reactions of the desired aminopyrazoles with enaminones or from a multicomponent step or one-pot reactions of diaminopyrazoles with triethyl orthoformate and diarylsulfonyl acetaldehydes by heating acetic acid under thermal or microwave conditions.[106] The antimicrobial activities of compounds **145a–d**, **145h**, **146a**, and **146c–h** (Fig. 7) were assessed by a disc diffusion method

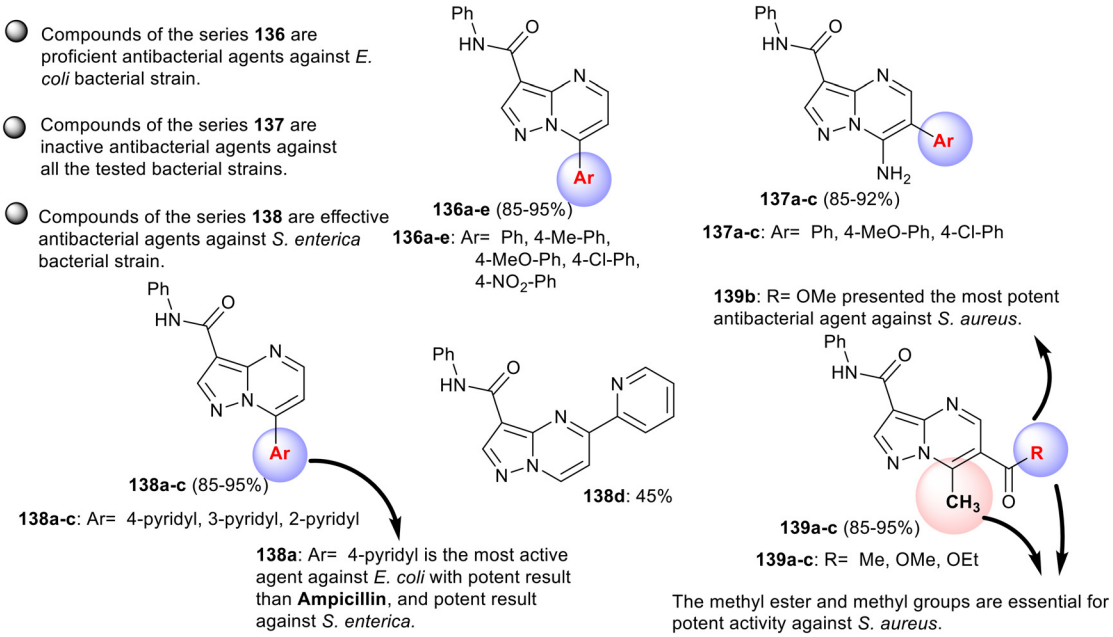
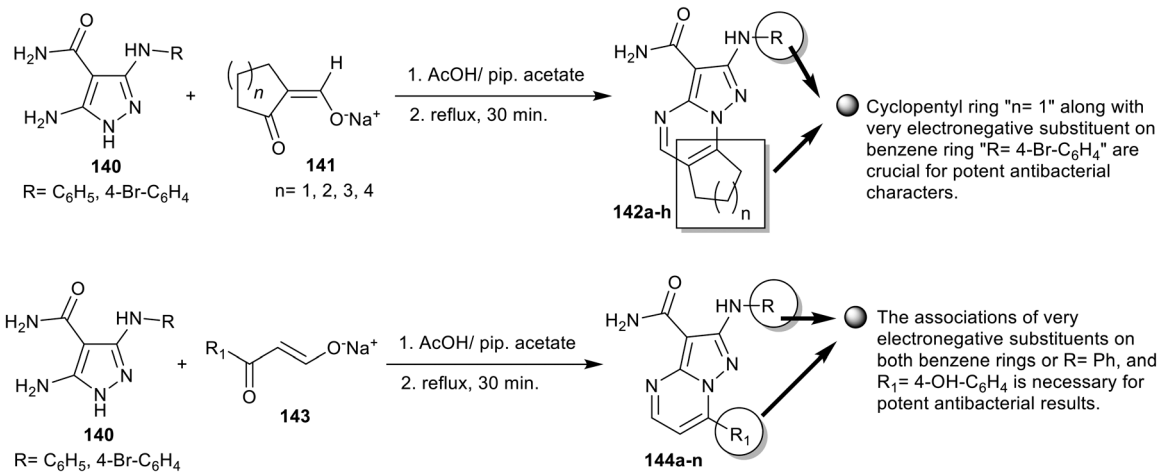


Fig. 5 SARs of bicyclic heterocycles as antibacterial agents.



Compounds	n	R	Compounds	R	R_1
142a	1	C_6H_5	**144d**	C_6H_5	C_6H_5
142b	1	4-Br-C_6H_4	**144e**	4-Br-C_6H_4	C_6H_5
142c	2	C_6H_5	**144f**	C_6H_5	4-Cl-C_6H_4
142d	2	4-Br-C_6H_4	**144g**	4-Br-C_6H_4	4-Cl-C_6H_4
142e	3	C_6H_5	**144h**	C_6H_5	4-Br-C_6H_4
142f	3	4-Br-C_6H_4	**144i**	4-Br-C_6H_4	4-Br-C_6H_4
142g	4	C_6H_5	**144j**	C_6H_5	4-OCH_3-C_6H_4
142h	4	4-Br-C_6H_4	**144k**	4-Br-C_6H_4	4-OCH_3-C_6H_4
Compounds	**R**	**R_1**	**144l**	C_6H_5	4-CH_3-C_6H_4
144a	CH_3	C_6H_5	**144m**	4-Br-C_6H_4	4-CH_3-C_6H_4
144b	C_2H_5	C_6H_5	**144n**	C_6H_5	4-OH-C_6H_4
144c	C_2H_5	4-Br-C_6H_4			

Fig. 6 The SARs of antibacterial pyrazolopyrimidines.

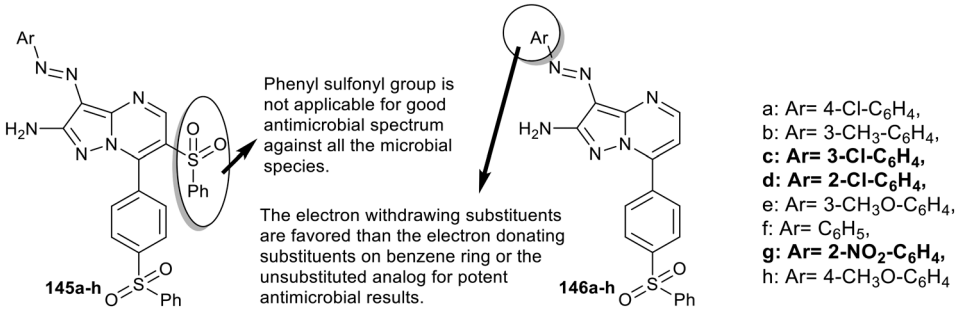


Fig. 7 The SARs of pyrazolopyrimidines as potent antimicrobial agents.

against fungal species (*A. niger* and *G. candidum*), Gram-positive bacterial species (*S. aureus*, *S. epidermidis*, *B. subtilis*, and *S. pyogenes*), and Gram-negative bacterial species (*P. aeruginosa*, *E. coli*, *K. pneumoniae*, and *S. typhimurium*). The results declared that compounds **146c** (inhibition zone diameter = 25.1 ± 1.2 mm) and **146g** (26.3 ± 0.63 mm) presented more potent antifungal activities than amphotericin B (23.3 ± 0.58 mm) against *A. niger*, while compound **146d** (25.2 ± 1.2 mm) has the same result as the antibiotic against *G. candidum*. Alternatively, the most effective potency was recorded for compounds **146c** (23.4 ± 0.63 mm) against *S. aureus*, **146d** (22.6 ± 0.72 mm) against *S. epidermidis*, **146f** (26.5 ± 0.58 mm) against *B. subtilis*, and inactive potency against *S. pyogenes*. For Gram-negative species, compounds **146d** (25.7 ± 1.2 mm) and **146g** (25.5 ± 1.2 mm) have more potent activities against *E. coli* compared to gentamicin (25.4 ± 1.2 mm), compound **146d** (26.6 ± 1.2 mm) against *K. pneumoniae*, and compounds **146c** (26.3 ± 0.58 mm), **146d** (26.2 ± 0.58 mm), and **146g** (26.6 ± 0.72 mm) against *S. typhimurium*, whereas these molecules are inactive against *P. aeruginosa*. Compounds **146c**, **146d**, and **146g** revealed the minimum inhibitory concentrations of 0.49–31.25 μg mL^{-1} in ranges matched with those of the antibiotic standards.

In addition, pyrazolopyrimidines were synthesized recently by Fouda *et al.*[107] in remarkable yields, and after a short reaction time *via* reactions of the corresponding diaminopyrazole with unsaturated nitriles in ethanol/pyridine mixture under thermal or microwave-assisted or sonication conditions. These compounds (Fig. 8) were appraised as antimicrobial agents against *S. aureus*, *B. subtilis*, and *S. mutans* (Gram-positive bacteria), *E. faecalis*, *P. vulgaris*, and *E. coli* (Gram-negative bacteria), and *A. fumigates*, *A. flavus*, and *C. albicans* using the disc diffusion assay at concentrations of 5 mg mL^{-1}. Gentamycin and ketoconazole were applied as standard antibiotics for bacterial and fungal species, respectively. Compound **150b** revealed remarkable activities against *E. faecalis* (25 mm) and *P. vulgaris* (40 mm) along with good activities against all other microbial species with inhibition zones ranging from 13 to 25 mm. In addition, compound **150a** presented insignificant antibacterial activities against the tested bacterial species (inhibition zones = 9–18 mm) along with no antifungal activities. Compounds **147a**, **147b**, **148a**, **148b**, **149a**, **149b**, **151a**, **151b**, and **152** revealed no antimicrobial activities in all cases except for compounds **147a**, **147b**, and **148b** that revealed moderate activities against *E. coli* (10 mm), *B. subtilis* (8 mm), and *S. aureus* (10 mm), respectively.

Sheikhi-Mohammareh *et al.*[108] have developed the synthesis of tetracyclic systems **155a–i** by gently heating carbonitrile **153** with 2-amino-*N*-substituted-benzamides **154** in DMF catalyzed by potassium carbonate (Scheme 22). The tetracyclic systems were evaluated as antibacterial agents against *K. pneumoniae* and *E. coli* at seven concentrations of serial dilutions of each sample (15.6–1000 ppm). The lower

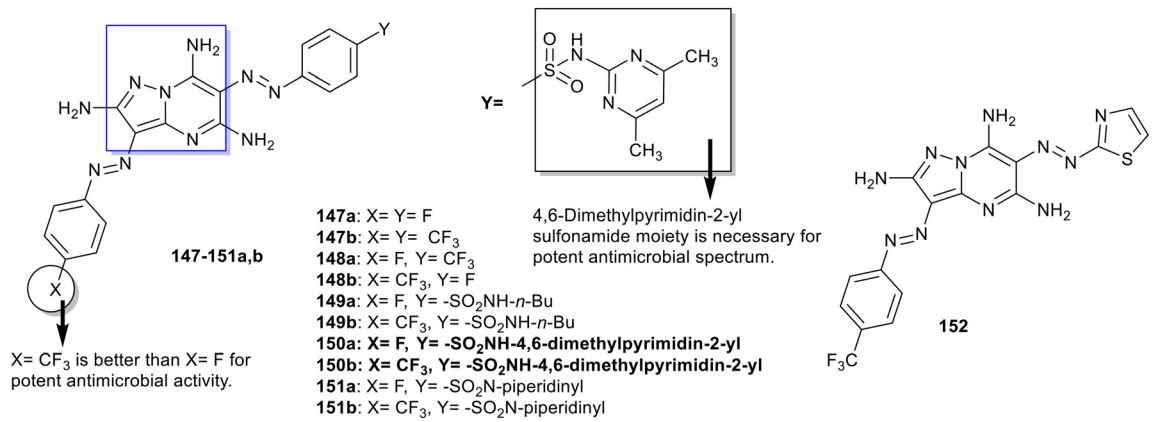
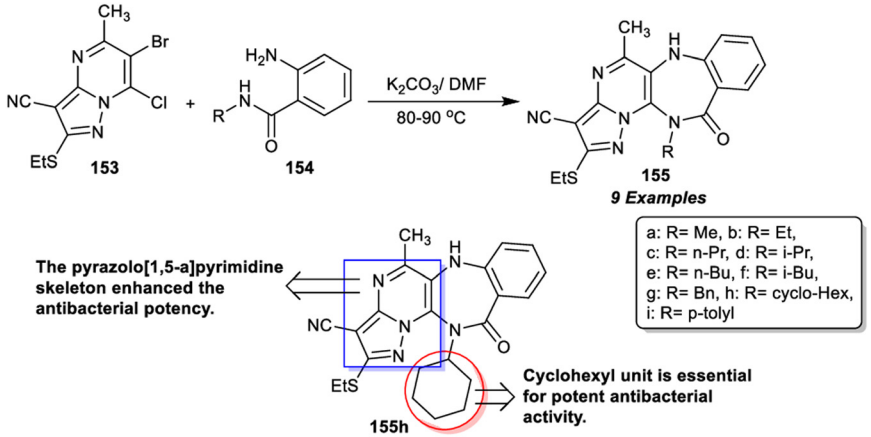


Fig. 8 The SARs of pyrazolopyrimidines as antimicrobial and anticancer agents.



Scheme 22 Simple synthesis of potent antibacterial agents.

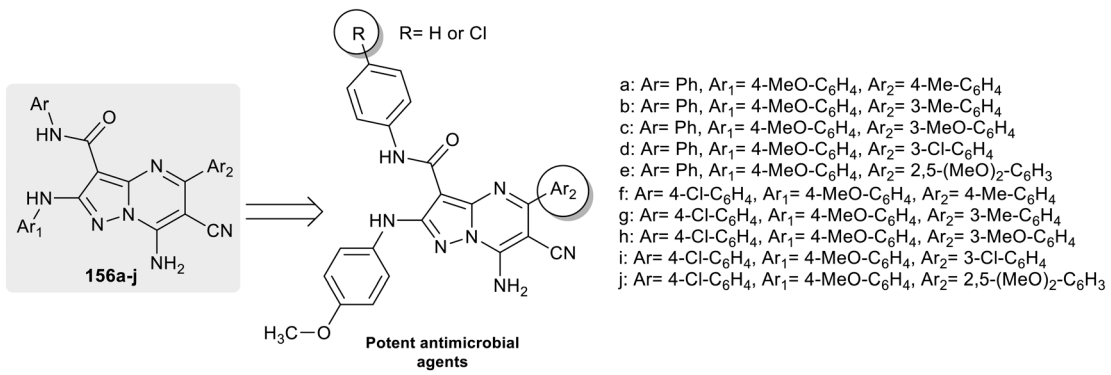


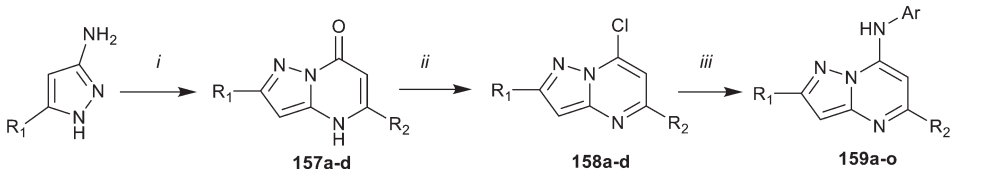
Fig. 9 The structures of substituted bicyclic heterocycles as potent antimicrobial agents.

concentrations of the samples revealed good efficiency to decrease the growth of the strains relative to clinical ones. Compound **155h** was found as the lone compound that can impede the growth of multi-drug-resistant hospital bacteria isolated from UTI victims with 100% growth inhibitory activity on both categories of bacterial species.

A series of pyrazolopyrimidines **156a–j** (Fig. 9) were efficiently synthesized by reactions of the corresponding aminopyrazoles with arylidene malononitriles in ethanol containing a catalytic amount of trimethylamine under heating conditions.[109] Accordingly, these compounds were evaluated by *in vitro* assay as antimicrobial agents against a diversity of microbial species. Thus, compounds **156b**, **156e** (IZ = 20 mm), and **156j** (IZ = 21 mm) are the most potent agents against *B. subtilis* species: however, the same compounds are the most active agents with inhibition zone diameters of 16 mm against *E. coli* species. On the other hand, compounds **156d**, **156e**, **156f**, **156g**, and **156i** introduced improved antifungal activities against *A. niger* species with inhibition zone diameters ranging from 15 to 18 mm. Also, most of the compounds are potent antifungal agents against *C. albicans* species; in particular, compound **156f** is the most effective agent (IZ = 18 mm) compared to cycloheximide (32 mm). Lately, Metwally *et al.*[110] have *in vitro* estimated the antibacterial potency of the proficiently synthesized pyrazolopyrimidines under bio-catalytic conditions using pepsin by applying a green protocol.

4.2. Antimalarial activity

Azeredo *et al.*[111] have investigated the preparation of pyrazolopyrimidines **159a–o** (Scheme 23) by reactions of aminopyrazoles with 1,3-diketones, followed by chlorination with $POCl_3$ and nucleophilic substitution of the chlorine atom with aryl amines in multi-step reactions. The compounds **159a–o** were assessed against *P. falciparum* (W2 clone, chloroquine-resistant) for cytotoxicity in BGM cells and percent inhibition of the P*f*DHODH enzyme. The results revealed that thirteen compounds have *in vitro* anti-*P. falciparum* activity against chloroquine-resistant parasites (IC_{50} = 1.2–92.4 μM). Compounds with 2-naphthyl substituents (Ar = 2-naphthyl) (**159d:** R_1 = CF_3, R_2 = CH_3, **159j:** R_1 = R_2 = CH_3, **159o:** R_1 = CH_3, R_2 = CF_3) and 3,5-dimethoxyphenyl (**159m:** R_1 = CH_3, R_2 = CF_3) at the 7-position exhibited the most potent activities. The *in vitro* and inhibitory percentage of P*f*DHODH indicated that compounds **159a** (Ar = 4-Cl-Ph, R_1 = CF_3, R_2 = CH_3), **159b** (Ar = 3,5-$(MeO)_2$-Ph, R_1 = CF_3, R_2 = CH_3), **159d** (Ar = 2-naphthyl, R_1 = CF_3, R_2 = CH_3), **159e** (Ar = 4-CF_3-Ph, R_1 = CF_3, R_2 = CH_3), **159h** (Ar = 3,5-$(MeO)_2$-Ph, R_1 = R_2 = CH_3), **159j** (Ar = 2-naphthyl, R_1 = R_2 = CH_3), **159k** (Ar = 4-CF_3-Ph, R_1 = R_2 = CH_3) and **159o** (Ar = 2-naphthyl, R_1 = CH_3, R_2 =



Reagents, and conditions:
(i) ethyl acetoacetate or ethyl 4,4,4-trifluoroacetoacetate, AcOH, 5-16 h, reflux, 71-93 °C ;
(ii) $POCl_3$, reflux, 1-4 h;
(iii) appropriate amine, EtOH or toluene, 25 °C, 14-26 h.

R_1= R_2= CF_3, CH_3
Ar= 2-naphthyl or aryl
15 Examples
44-92% yields

Scheme 23 Synthesis of pyrazolopyrimidines.

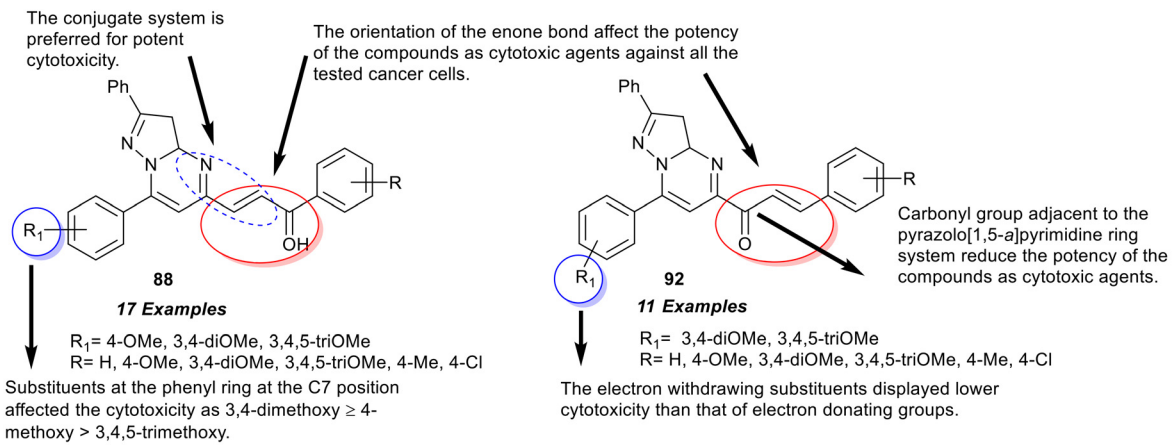
CF_3) have the maximum percentage inhibitions. Other recent research reported the use of 8-aminoquinoline-pyrazolopyrimidines and 7-arylaminopyrazolopyrimidines as antimalarial agents.[112] Similar recent reports are related to the evaluation of the biological potency of pyrazolopyrimidines or their isomeric structures as anti-*P. falciparum* agents.[113,114]

4.3. Antiproliferative activity

The cytotoxicity of chalcone-based pyrazolopyrimidines **88** and **92** was appraised using an MTT assay against A549, MDA-MB-231, DU-145, and HEK293 cells.[97] The results showed potent activities relative to the reference standard, erlotinib, with inhibitive potentials ranging from IC_{50} = 2.6 μM to 34.9 μM. In brief, compounds of analogs **88** revealed more potent cytotoxicity than the compounds of analogs **92**. Both series have the same structure except for the orientation of the enone bond, in which the carbonyl group adjacent to the pyrazolopyrimidine ring system reduces the potency of the compounds as cytotoxic agents. The most potent analogs are compounds **88b** (R_1 = 4-OMe, R = 4-OMe), **88h** (R_1 = 3,4-diOMe, R = 3,4-diOMe), and **88i** (R_1 = 3,4-diOMe, R = 3,4,5-triOMe) with high efficiency in inhibiting the growth of cancer cells along with low cytotoxicity against human embryonic kidney (HEK293) cells. The SARs of both series in Fig. 10 presented the effect of the position of the enone motif linked to the pyrazolopyrimidine scaffold and the nature of substituents on the phenyl ring on the potency of the molecules as cytotoxic agents.[97]

4.4. Cytotoxic activity

Kamal *et al.*[115] have deliberated the synthesis of benzoylpiperazinyl methanones **160a–x** by condensation of 2-phenyl-7-(substituted-aryl)pyrazolo[1,5-*a*]pyrimidine-5-carboxylic acids with piperazin-1-yl(2-((aryl-methyl)amino)-phenyl)methanones in dichloromethane under catalytic conditions of EDCI/HOBt by stirring at 0 °C to room temperature. The respective pyrazolo[1,5-*a*]pyrimidinyl amides **160a–x** were assessed *in vitro* as anticancer agents using the



Compounds	IC_{50} (mM)			
	A549	MDA-MB-231	DU-145	HEK293
88b	2.9±0.3	6.3±0.3	8.5±0.4	36.1±0.9
88h	3.9±0.4	2.6±0.6	7.2±0.4	32.5±0.7
88i	7.2±0.4	4.7±0.3	8.3±0.3	35.0±1.2

Fig. 10 The SARs of the most potent cytotoxic agents.

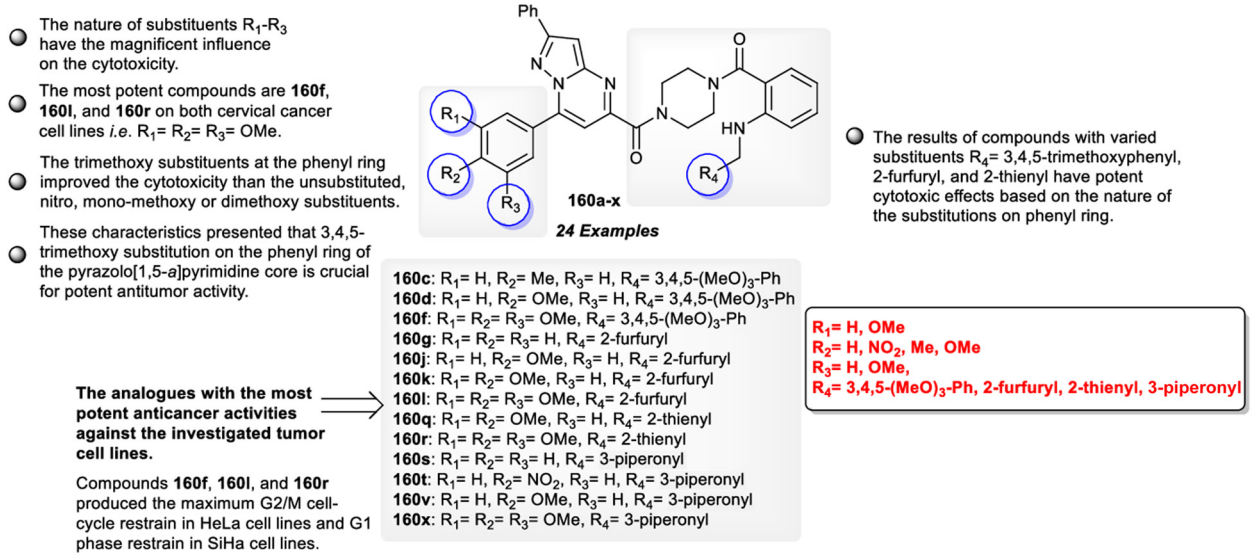


Fig. 11 The SARs of carboxamides as potent cytotoxic agents.

MTT assay on human cervical cancer, *i.e.* HeLa and SiHa cell lines, in which roscovitine was used as a standard anticancer drug. The results verified that compound **160r** is the most active agent on SiHa cells with IC_{50} = 1.56 ± 0.30 μM; thereafter, compound **160l** has the second strongest influence with IC_{50} = 1.61 ± 0.30 μM, **160q** has the second strongest potency with IC_{50} = 1.81 ± 0.22 μM, and compounds **160f**, **160g**, and **160t** with IC_{50} = 1.91 ± 0.17, 1.93 ± 0.32, and 1.99 ± 0.17 μM, respectively. All these compounds have higher potency than the anticancer standard, roscovitine (IC_{50} = 2.69 ± 0.35 μM). The rest of the compounds demonstrated good cytotoxicity (IC_{50} = 2.04 ± 0.48 to 2.71 ± 0.12 μM) relative to the standard anticancer on SiHa cell lines. On the other hand, compounds **160c** (IC_{50} = 1.81 ± 2.24 μM), **160d** (IC_{50} = 1.89 ± 0.13 μM), **160f** (IC_{50} = 1.51 ± 0.20 μM), **160j** (IC_{50} = 1.73 ± 0.18 μM), **160k** (IC_{50} = 1.56 ± 0.12 μM), **160l** (IC_{50} = 1.54 ± 0.18 μM), **160q** (IC_{50} = 1.99 ± 0.17 μM), **160r** (IC_{50} = 1.46 ± 0.15 μM), **160s** (IC_{50} = 1.95 ± 0.19 μM), **160t** (IC_{50} = 1.99 ± 0.64 μM), **160v** (IC_{50} = 1.95 ± 0.42 μM), and **160x** (IC_{50} = 1.76 ± 0.24 μM) exhibited the most potent cytotoxicity on the HeLa cell line, higher than that of roscovitine (IC_{50} = 2.20 ± 0.12 μM). The SARs of the tested compounds as shown in Fig. 11 demonstrated that the substitution of the phenyl ring (R_1–R_3) has a great impact on the anticancer potency of the compounds, but the most effective core is related to the pyrazolo[1,5-*a*]pyrimidine skeleton based on the reasonable influences of most of the compounds relative to the anticancer standard.

Kamal *et al.*[7b] have prepared carboxamide analogs **161a–t** (Fig. 12) by condensation of 2-phenyl-7-(substituted-aryl)pyrazolo[1,5-*a*]pyrimidine-5-carboxylic acids with 5,6-disubstituted-benzo[*d*]thiazol-2-amines in dichloromethane under the same preceding conditions (EDCI/HOBt, CH_2Cl_2, 0 °C → rt, 8 h). The anticancer activity of carboxamides **161a–t** was evaluated *in vitro* by the MTT assay on various tumor cells, *i.e.* lung (A549), prostate (DU-145), breast (MCF-7), renal

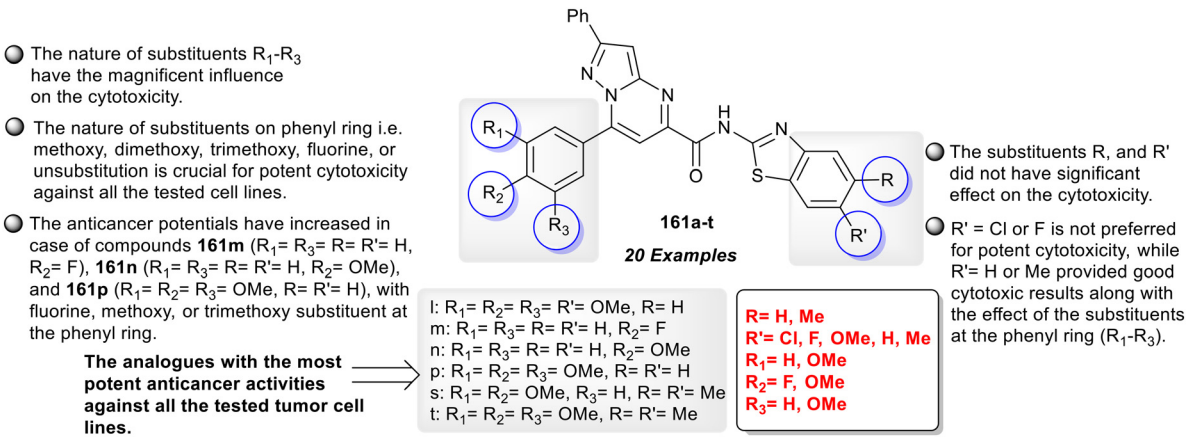
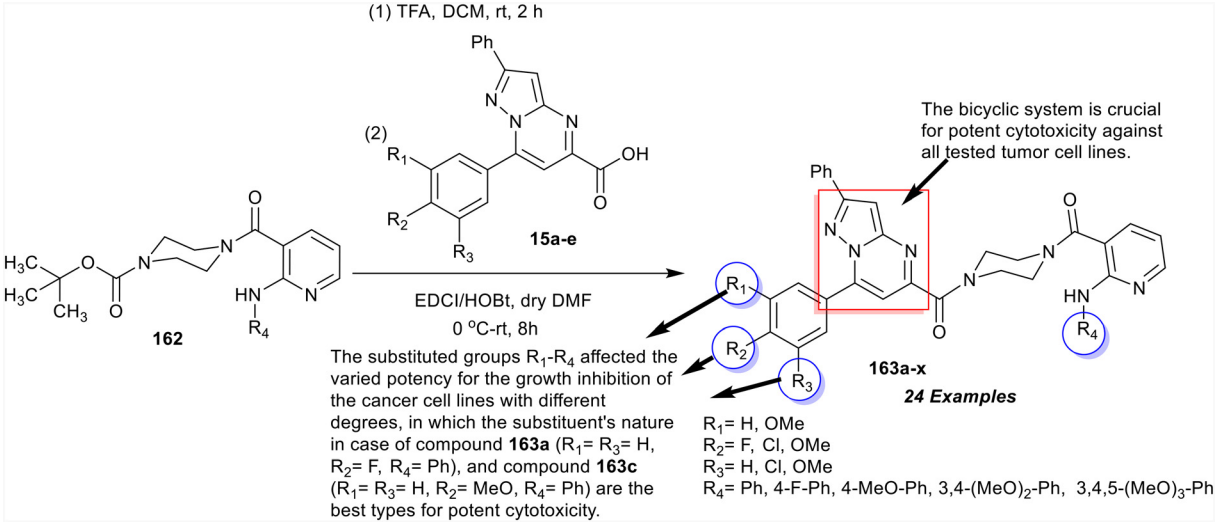


Fig. 12 The SARs of carboxamides as potent anticancer agents.



Scheme 24 SARs of diamides as potent cytotoxic agents.

cell carcinoma (ACHN), cervical (HeLa), using roscovitine as an anticancer standard. The results verified that compounds **161m**, **161n**, and **161p** exhibited the most potent cytotoxicity with IC_{50} = 1.94, 1.54, and 2.01 μM, respectively, while compounds **161l**, **161s**, and **174t** are strong cytotoxic agents with slightly lower efficiency on lung cell lines (A549) relative to the anticancer standard (IC_{50} = 2.18 μM). The most potent cytotoxic agents were found to be **174m**, **174n**, and **174p** with IC_{50} = 2.08, 2.95, and 3.16 μM, respectively, on the prostate cell line DU-145; the less potency of the same compounds on this cancer cell line is due to the nature of the tumor cell that affects the obtained results. Compounds **161l**, **161m**, **161n**, **161p**, **161s**, and **161t** revealed more potent anticancer activities with IC_{50} = 2.95, 2.29, 2.23, 2.88, 2.51, and 2.29 μM, respectively, than the anticancer standard (IC_{50} = 3.98 μM) on the breast cancer cell line MCF-7. The most potent cytotoxic agent for renal cell carcinoma (ACHN) was compound **161n** (IC_{50} = 1.69 μM), while compound **161m** (IC_{50} = 2.63 μM) was the strongest cytotoxic agent for cervical (HeLa) cell lines. On the other hand, compounds **161a–k** exhibited good to moderate cytotoxic activities on all the tumor cell lines. Fig. 12 presented the SARs of the tested compounds as potent antitumor agents.[7b]

Kamal *et al.*[76] reported the synthesis of diamides **163a–x** (Scheme 24) by coupling carboxylic acids **15** with *tert*-butyl carboxylates **162** through two step-synthesis. The first step involved the acid cleavage with the removal of the Boc-protection on the piperazine core of compounds **162** followed by the coupling process in the second step. The compounds were evaluated as anticancer agents against SiHa, MCF-7, HeLa, and IMR-32 tumor cell lines by the MTT assay. The results indicated that compounds **163a** (R_1 = R_3 = H, R_2 = F, R_4 = Ph) and **163c** (R_1 = R_3 = H, R_2 = MeO, R_4 = Ph) were the most effective among this series against the tested cell lines. Generally, compounds **163a**, **163c**, **163j** (R_1 = R_2 = R_3 = MeO, R_4 = 4-F-Ph), **163m** (R_1 = R_3 = H, R_2 = MeO, R_4 = 4-MeO-Ph), **163o** (R_1 = R_2 = R_3 = MeO, R_4 = 4-MeO-Ph), **163q** (R_1 = H, R_2 = R_3 = Cl, R_4 = 3,4-$(MeO)_2$-Ph), **163r** (R_1 = R_3 = H, R_2 = MeO, R_4 = 3,4-$(MeO)_2$-Ph), **163s** (R_1 = H, R_2 = R_3 = OMe, R_4 = 3,4-$(MeO)_2$-Ph), **163t** (R_1 = R_2 = R_3 = MeO, R_4 = 3,4-$(MeO)_2$-Ph) and **163w** (R_1 = R_3 = H, R_2 = MeO, R_4 = 3,4,5-$(MeO)_3$-Ph) revealed potent cytotoxicity to all tumor cells relative to the results of the reference standard, doxorubicin. Compound **163a** presented the highest effective cytotoxic influence on the verified tumor cell lines with IC_{50} = 2.65, 1.79, 2.29, and 4.65 μM, respectively. Compound **6c** exhibited a second-order

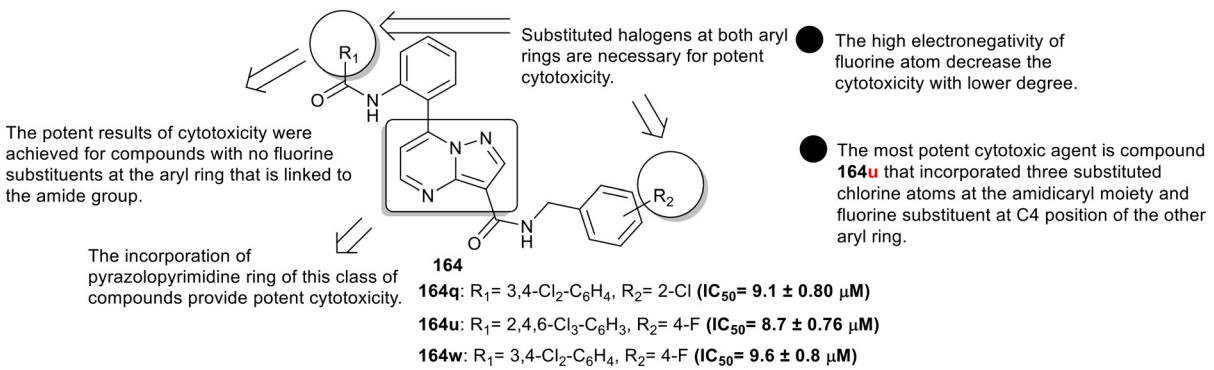
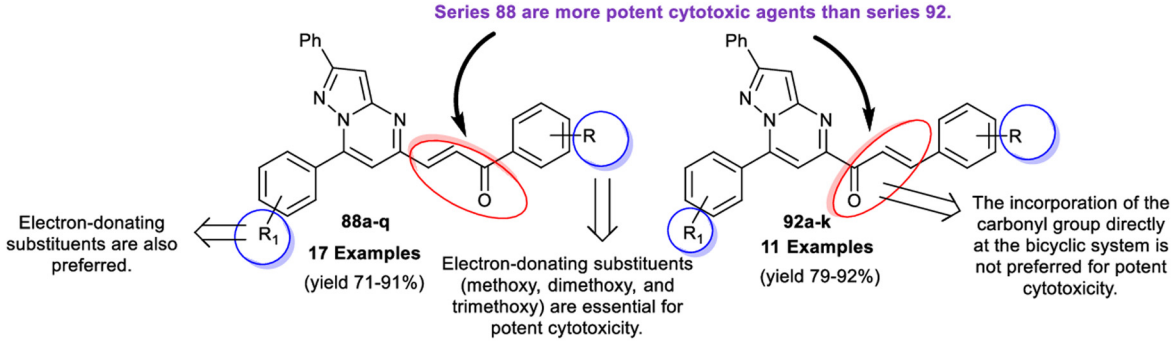


Fig. 13 The SARs of diamides of pyrazolopyrimidines as cytotoxic agents.



Compounds	R	R_1	Compounds	R	R_1
88a	H	4-OMe	**88o**	3,4-diOMe	3,4,5-triOMe
88b	4-OMe	4-OMe	**88p**	3,4,5-triOMe	3,4,5-triOMe
88c	3,4-diOMe	4-OMe	**88q**	4-Cl	3,4,5-triOMe
88d	H	3,4-diOMe	**92a**	H	3,4-diOMe
88e	4-Me	3,4-diOMe	**92b**	4-Me	3,4-diOMe
88f	3,4-diMe	3,4-diOMe	**92c**	4-OMe	3,4-diOMe
88g	4-OMe	3,4-diOMe	**92d**	3,4,5-triOMe	3,4-diOMe
88h	3,4-diOMe	3,4-diOMe	**92e**	4-Cl	3,4-diOMe
88i	3,4,5-triOMe	3,4-diOMe	**92f**	H	3,4,5-triOMe
88j	4-Cl	3,4-diOMe	**92g**	4-Me	3,4,5-triOMe
88k	H	3,4,5-triOMe	**92h**	4-OMe	3,4,5-triOMe
88l	4-Me	3,4,5-triOMe	**92i**	3,4-diOMe	3,4,5-triOMe
88m	3,4-diMe	3,4,5-triOMe	**92j**	3,4,5-triOMe	3,4,5-triOMe
88n	4-OMe	3,4,5-triOMe	**92k**	4-Cl	3,4,5-triOMe

Fig. 14 The SARs of the most effective cytotoxic agents.

cytotoxic capacity with IC_{50} = 3.33, 2.16, 2.43, and 4.75 μM, respectively, against all the tested tumor cell lines (Scheme 24).

Compounds **164** were tested as anticancer agents using the MTT assay against HeLa with cisplatin as a reference standard. Compounds **164q**, **164u**, and **164w** exhibited potent cytotoxicity with IC_{50} less than 10 μM and were considered more potent than cisplatin (IC_{50} = 17.83 μM) used as an anticancer drug. The SARs of the tested diamides **164** are presented in Fig. 13.[95]

Bagul *et al.*[97] have reported the synthesis of two series of the relevant pyrazolopyrimidinyl chalcones; one of them (series **88a–q**) was prepared by the reactions of β-ketoesters with 3-amino-5-phenyl-pyrazole in boiling ethanol in acid medium, followed by reductive cleavage of the formed esters to the aldehydes, which condensed with active methylenes to yield series **88a–q**. The other series **92a–k** was prepared through the transformation of the previously prepared aldehydes in the last series to the respective acetyl analogs, which reacted with aryl aldehydes by condensation catalyzed by barium hydroxide in methanol at room temperature. The molecules of the two series were assessed as anticancer agents against A549, MDA-MB-231, and DU-145 tumor cells using an MTT assay (Fig. 14).

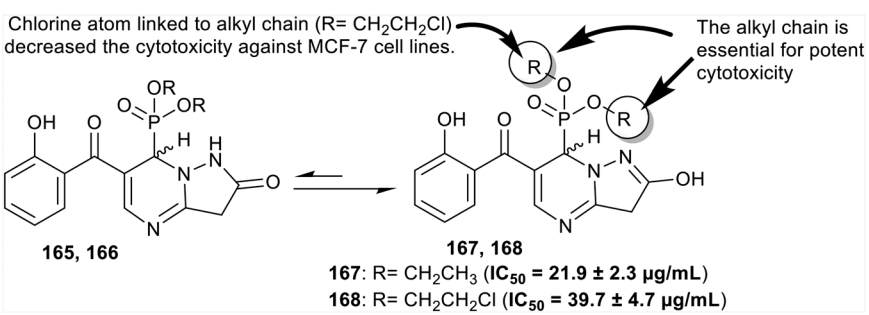


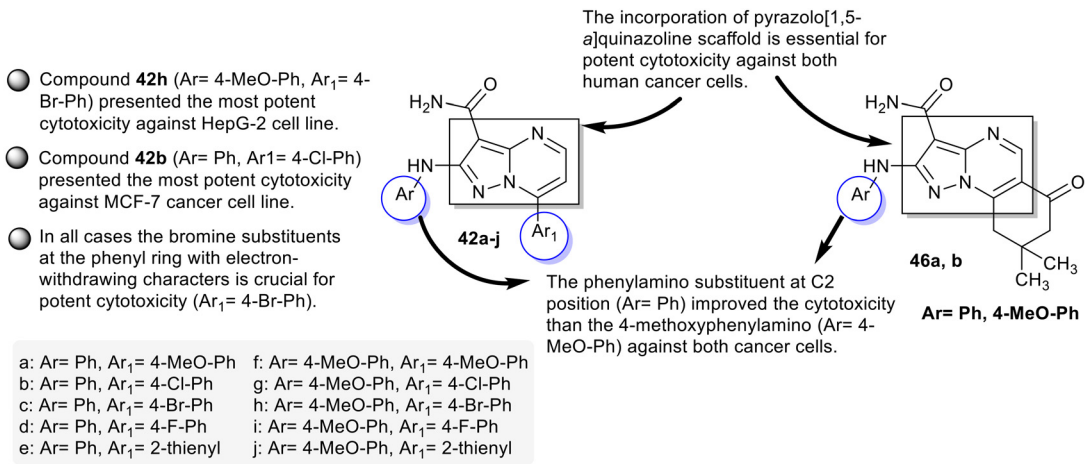
Fig. 15 The SARs of dialkyl phosphonates as anticancer agents.

Compounds **88a–c** (8.6, 2.9, 7.4 μM), **88f** (9.3 μM), **88h** (3.9 μM), and **88i** (7.2 μM) revealed the strongest cytotoxicity on A549 cell line than erlotinib (10.39 μM). In addition, compounds **88a–d** (9.9, 6.3, 8.7, 11.8 μM), **88f** (9.3 μM), **88f–i** (11.5, 13.9, 2.6, 4.7 μM), **88m** (13.5 μM), **88n** (13.2 μM) and **92i** (14.19 μM) revealed the most potent cytotoxicity to the MDA-MB-231 cell line than the standard erlotinib (14.74 μM). Compounds **88a–d** (13.7, 8.5, 16.4, 10 μM), **88f** (9.3 μM), **88f–i** (12.1, 14.6, 7.2, 8.3 μM), **88m** (15.4 μM), **92a–d** (15.57, 19.49, 14.93, 14.74 μM), **92f** (16.31 μM), **92h** (16.91), **92i** (15.33), and **92j** (17.91 μM) revealed the most potent cytotoxicity to the DU-145 cell line in comparison to **erlo**tinib (18.4 μM). The substitution of the phenyl ring with one or two methoxy groups (R_1 = OMe or diOMe) is preferred for potent anticancer potency. Generally, the substituents at the phenyl ring attached to the chalcone moiety (R = 4-OMe, 3,4-diOMe and 3,4,5-triOMe) are perfect for potent cytotoxicity than the unsubstituted or substituted electronegative substituents at the phenyl rings. Series **88** are more potent cytotoxic agents than series **92**, indicating the role of the chalcone moiety and the effect of linkage position (Fig. 14).[97]

The compounds **167** and **168** (Fig. 15) were tested as anticancer agents on the MCF-7, HepG2, HCT116, and PC3 cell lines by the SRB assay using tamoxifen as a scale standard. The molecules, in general, showed no activities against the HCT116 and PC3 cell lines. In addition, compound **167** is more potent with IC_{50} = 21.9 ± 2.3 μg mL^{-1} than compound **168** that has IC_{50} = 39.7 ± 4.7 μg mL^{-1}. Compounds **167** and **168** in general are less potent than tamoxifen (IC_{50} = 8.50 ± 0.90 μg mL^{-1}). The influence of the chlorine atom decreased the potency of **168** against the MCF-7 cell lines.[116]

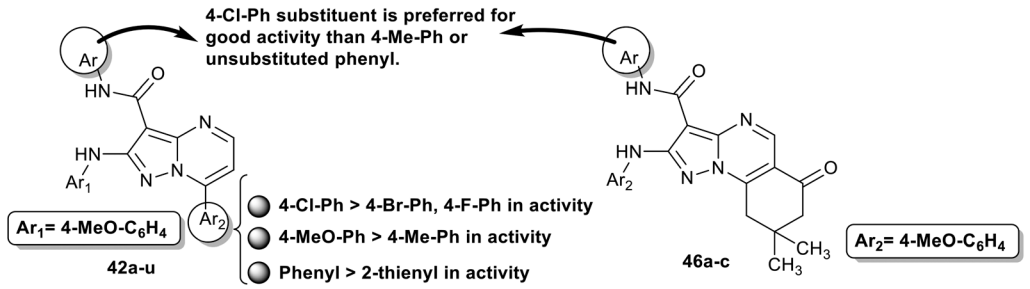
A series of bicyclic and tricyclic carboxamides **42** and **46** were evaluated as anticancer agents against HepG-2 and MCF-7 tumor cells by employing the MTT assay. The carboxamide **42h** is the greatest cytotoxic agent to the HepG-2 cell line (IC_{50} = 70.3 ± 4.1 μg mL^{-1}) compared to doxorubicin (IC_{50} = 80.9 ± 2.1 μg mL^{-1}). In general, all the compounds of these series presented strong cytotoxicity to both cancer cells. Compounds **42c** and **46a** recorded the second order of potency on the HepG-2 cell line (IC_{50} = 76.2 ± 3.9 and 77.6 ± 4.3 μg mL^{-1}). Alternatively, compounds **42a–c** offered the greatest cytotoxicity to the MCF-7 cell line (IC_{50} = 63.4 ± 3.6, 63.2 ± 5.9, and 64.0 ± 2.8 μg mL^{-1}) compared to the reference standard (IC_{50} = 65.6 ± 4.2 μg mL^{-1}). The other compounds showed potent cytotoxicity equal to or near that of the reference standard (Fig. 16).[87]

El-Naggar *et al.*[86] have prepared two series of pyrazolopyrimidines *via* reactions of the aminopyrazoles with either acyclic enaminones or cyclic enaminones in acetic acid. The anticancer activity was assessed *in vitro* on HepG-2 and MCF-7 tumor cell lines employing the MTT assay, in which doxorubicin was used as a specific anticancer agent. The results revealed that compounds **42c**, **42d**, **42h**, **42j**, **42k**, **42l**, **42o**, **42q**, **42r**, **42s**, **42t**, and **46c** (Fig. 17) (IC_{50} = 72.2–79.5 μM) exhibited marginally higher cytotoxic activities than doxorubicin (IC_{50} = 80.9 ± 2.1 μM) on HepG-2 cancer cells. Additionally, compounds **42a**, **42b**, **42c**, **42j**, and **42u**



Compounds	HepG-2	MCF-7
42a	79.8 ± 6.1	63.4 ± 3.6
42b	86.6 ± 3.5	**63.2 ± 5.9**
42c	76.2 ± 3.9	64.0 ± 2.8
42h	**70.3 ± 4.1**	65.3 ± 3.1
46a	77.6 ± 4.3	66.5 ± 1.9
Doxorubicin	80.9 ± 2.1	65.6 ± 4.2

Fig. 16 SARs of bicyclic and tricyclic pyrazolo[1,5-*a*]quinazoline-3-carboxamides as potent cytotoxic agents.



Compounds	Ar	Ar1	Compounds	Ar	Ar1
42a	C_6H_5	C_6H_5	**42m**	4-CH_3-C_6H_4	4-F-C_6H_4
42b	C_6H_5	4-CH_3-C_6H_4	**42n**	4-CH_3-C_6H_4	2-thienyl
42c	C_6H_5	4-CH_3O-C_6H_4	**42o**	4-Cl-C_6H_4	C_6H_5
42d	C_6H_5	4-Cl-C_6H_4	**42p**	4-Cl-C_6H_4	4-CH_3-C_6H_4
42e	C_6H_5	4-Br-C_6H_4	**42q**	4-Cl-C_6H_4	4-CH_3O-C_6H_4
42f	C_6H_5	4-F-C_6H_4	**42r**	4-Cl-C_6H_4	4-Cl-C_6H_4
42g	C_6H_5	2-thienyl	**42s**	4-Cl-C_6H_4	4-Br-C_6H_4
42h	4-CH_3-C_6H_4	C_6H_5	**42t**	4-Cl-C_6H_4	4-F-C_6H_4
42i	4-CH_3-C_6H_4	4-CH_3-C_6H_4	**42u**	4-Cl-C_6H_4	2-thienyl
42j	4-CH_3-C_6H_4	4-CH_3O-C_6H_4	**46a**	C_6H_5	4-CH_3O-C_6H_4
42k	4-CH_3-C_6H_4	4-Cl-C_6H_4	**46b**	4-CH_3-C_6H_4	4-CH_3O-C_6H_4
42l	4-CH_3-C_6H_4	4-Br-C_6H_4	**46c**	4-Cl-C_6H_4	4-CH_3O-C_6H_4

Fig. 17 The SARs of anticancer pyrazolopyrimidines.

presented slightly higher cytotoxic effects than doxorubicin (IC_{50} = 65.6 ± 4.2 μM) on MCF-7 cell lines. The most efficient compound is **42o** (IC_{50} = 72.2 ± 3.8 μM) against HepG-2 cancer cells, while the most effective one on MCF-7 tumor cell lines was found to be compound **42a** (IC_{50} = 63.1 ± 3.1 μM).

The development and the progress of prostate cancer (PCa) were focused on in the past decades on applying the androgen receptor (AR), which has a serious role in contemporary androgen deprivation therapy. Nonsteroidal antiandrogens were effectively used for the treatment of prostate cancer, but with drug resistance after a year and a half. Wang *et al.*[117] have identified a combination of structure- and ligand-based methodologies to obtain potent androgen receptor antagonists. In this sequence, the rate of inhibition of PCa- and DHT-induced transcriptional activation of androgen receptors was evaluated *in vitro* for a series of pyrazolopyrimidines **169–172** (Fig. 18) to identify their potential antagonistic effects. Compound **171** showed

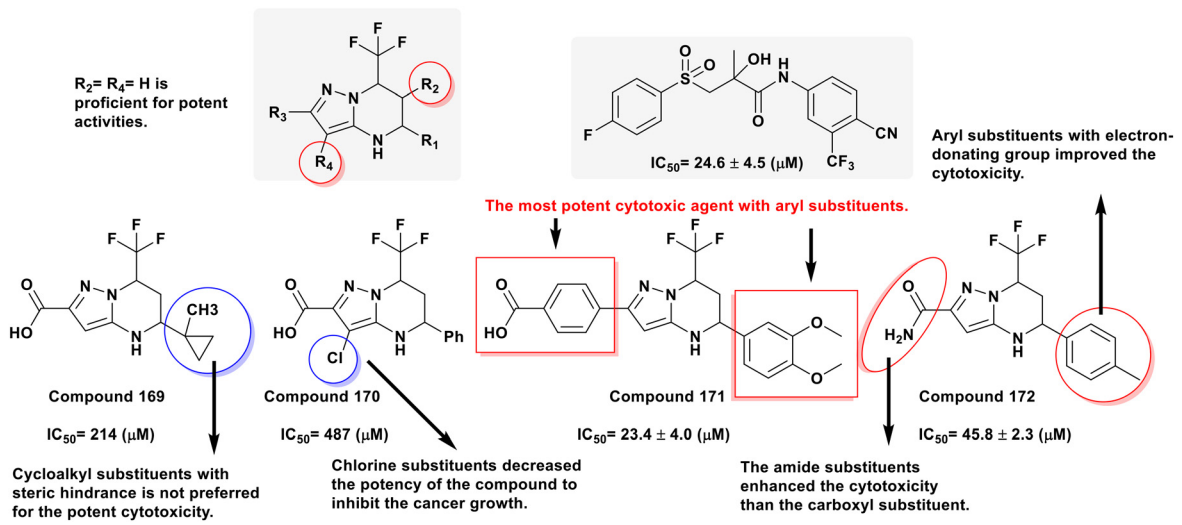


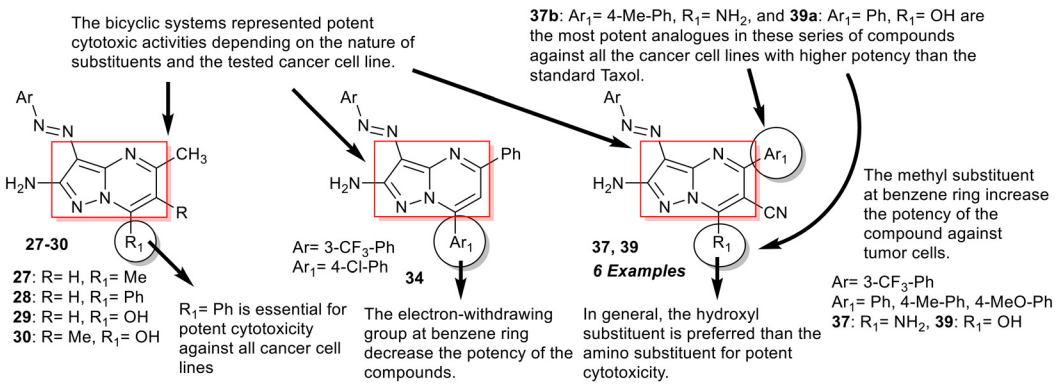
Fig. 18 SARs of the potent anticancer agents for prostate cancer cells.

potent results (IC_{50} = 23.4 ± 4.0 μM) compared to that of *R*-bicalutamide (IC_{50} = 24.6 ± 4.5 μM), while compound **172** exhibited a potential antagonistic effect with IC_{50} = 45.8 ± 2.3 μM in the second order of potency by applying a cell proliferation assay. The representative mechanism estimated that compound **171** prevented H12 in AR LBD from closing to distort the formation of AF2 with invalid transcription. The series of this class of heterocycles with an antiandrogenic scaffold functions as a core structure of an androgen receptor antagonist. It is worth mentioning that the substituents at the C2 position of bicyclic pyrazolopyrimidines are preferred to be aryl substituents with the carboxylic group at the *p*-position, while the substituents at the C2 position are better to be aryl substituents substituted with electron-donating groups for potent cytotoxicity.

The cytotoxic potency of pyrazolopyrimidines **147–151a** and **b** and **152** (Fig. 8) was evaluated *in vitro* by the MTT assay against tumor cells such as HepG-2, HCT-116, and MCF-7 cell lines. The results verified that compounds **147b** (0.3 ± 0.01 μg mL^{-1}), **150b** (4.5 ± 0.4 μg mL^{-1}), and **151b** (1.4 ± 0.03 μg mL^{-1}) have potent cytotoxicity to MCF-7 cells relative to doxorubicin (1.2 ± 0.2 μg mL^{-1}). In the case of the HepG-2 cell line, compounds **147b** (0.6 ± 0.2 μg mL^{-1}), **150b** (3.9 ± 0.4 μg mL^{-1}), **151b** (3.4 ± 0.6 μg mL^{-1}), and **152** (4.1 ± 0.3 μg mL^{-1}) have the most potent cytotoxicity (doxorubicin, IC_{50} = 0.9 ± 0.3 μg mL^{-1}). On the other hand, compounds **147b** (0.4 ± 0.02 μg mL^{-1}), **150b** (2.7 ± 0.6 μg mL^{-1}), and **151b** (2.4 ± 0.4 μg mL^{-1}) exhibited the most potent cytotoxicity to the HCT-116 cell line relative to doxorubicin (1.6 ± 0.2 μg mL^{-1}). Generally, compound **147b** is the most effective cytotoxic agent compared to the other compounds on the different tumor cells.[107]

Fayed *et al.*[80] reported the cytotoxic activity of a series of substituted pyrazolopyrimidines **27–30**, **34**, **37**, and **39** against HepG-2, HCT-116, and MCF-7 cell lines by the MTT assay. The results showed that the series of compounds **37** and **39** are the most potent cytotoxic agents against the HepG-2 cell line with IC_{50} = 0.64–24.8 μg mL^{-1}. The most potent analog is compound **37b** with IC_{50} = 0.64 μg mL^{-1}. Additionally, compounds **37b** and **39a** presented the highest efficiency in inhibiting the growth of the HCT-116 cancer cell lines with IC_{50} = 1.89 and 2.39 μg mL^{-1}, with more potent activity than the reference standard (2.9 μg mL^{-1}). Consistent results are recorded for the same compounds against the MCF-7 cell lines with IC_{50} = 2.79 and 3.09 μg mL^{-1}, with effectiveness higher than that of Taxol (4.7 μg mL^{-1}). The SARs was discussed as verified in Fig. 19.

Kaping *et al.*[91] have synthesized arylpyrazolopyrimidines **49–51**, **54**, and **56** through reactions of aminopyrazole with either enaminones or enaminonitriles in a water/ethanol mixture under $KHSO_4$ catalytic conditions. Compounds **50c** and **50d** were obtained as products from the reaction of aminopyrazole with 3-(dimethylamino)-1-(pyridin-2-yl)prop-2-en-1-one in 41% and 45% yield. The anticancer activities were evaluated for compounds **49–51**, **54**, and **56** thru the MTT assay on CHO K1 cell lines. Compound **50c** exhibited the most reduction in the color of MTT after exposure to the cancer cells relative to the unexposed cell lines. The order of decreased absorbance was found as follows, **54b**, **50a**, **56**,



Compounds	IC_{50} (μg/mL) HepG-2	IC_{50} (μg/mL) HCT-116	IC_{50} (μg/mL) MCF-7
35a	24.8	22.6	23.5
35b	0.64	1.89	2.79
35c	10.3	22.5	23.8
36a	2.41	2.39	3.09
36b	8.47	12.0	11.2
Taxol	4.3	2.9	4.7

Fig. 19 SARs of bicyclic pyrazolopyrimidines as potent cytotoxic agents.

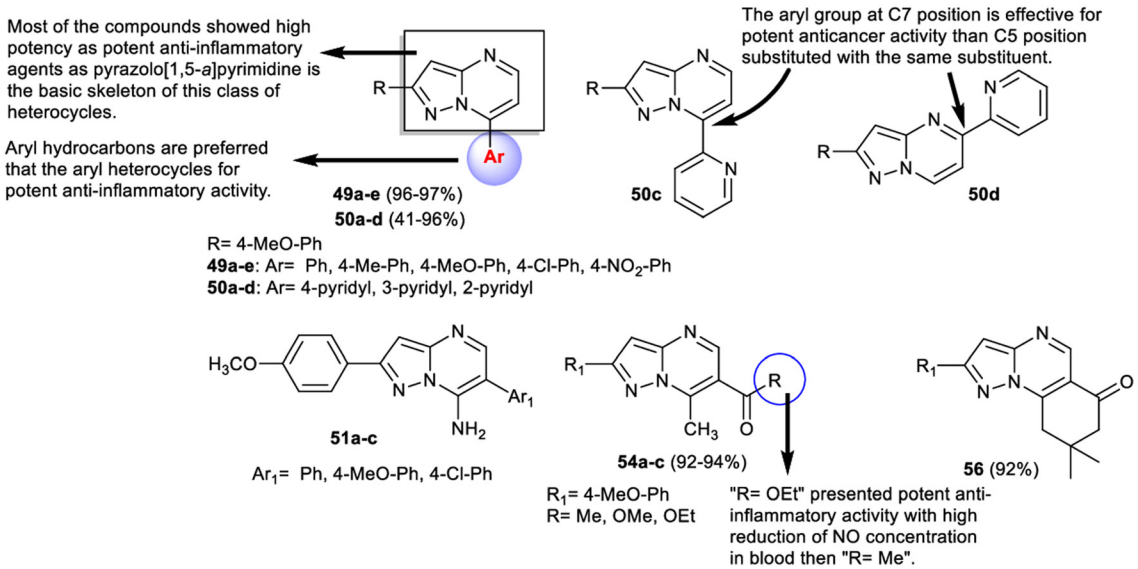


Fig. 20 The SARs of cytotoxic and anti-inflammatory agents.

49a, **50d**, **54c**, **51c**, **51b**, **49c**, **50b**, and **51a**, in which these compounds are believed to present remarkable cytotoxic potency on CHO K1 cell lines. The compounds of these series could be applied significantly on a large scale as cytotoxic agents against CHO K1 tumor cell lines. The SARs indicated that (1) the 2-aryl-pyrazolopyrimidine moiety is the most effective core that controls the cytotoxic effect. (2) The incorporation of pyridinyl substituents (compounds **50a–d**) provided the most potent cytotoxicity, in which 2-pyridyl is more effective than the other isomeric structures. (3) The electron-donating group (amino group of compounds **51a–c**) led to improved cytotoxicity. (4) The methyl ester substituent (compounds **54a–c**) is essential for potent cytotoxic effect compared to the ethyl ester analog or the acetyl one. (5) The tricyclic system (compound **56**) provided potent cytotoxicity (Fig. 20).

Kaping *et al.*[93] have synthesized compounds **173–177** starting from 3-amino-*N*-antipyrinyl-1*H*-pyrazole-4-carboxamide following their preceding synthetic procedure under the same conditions (in H_2O/EtOH, $KHSO_4$ acid medium under ultrasonic irradiation, at 60 °C).[87] The cytotoxic effects of the series of pyrazolopyrimidines **173–177** were assessed by MTT colorimetric assay on CHO K1 cells. It was found that compounds **173a**, **173b**, **174a**, **174b**, **177a**, and **177b** reduced the

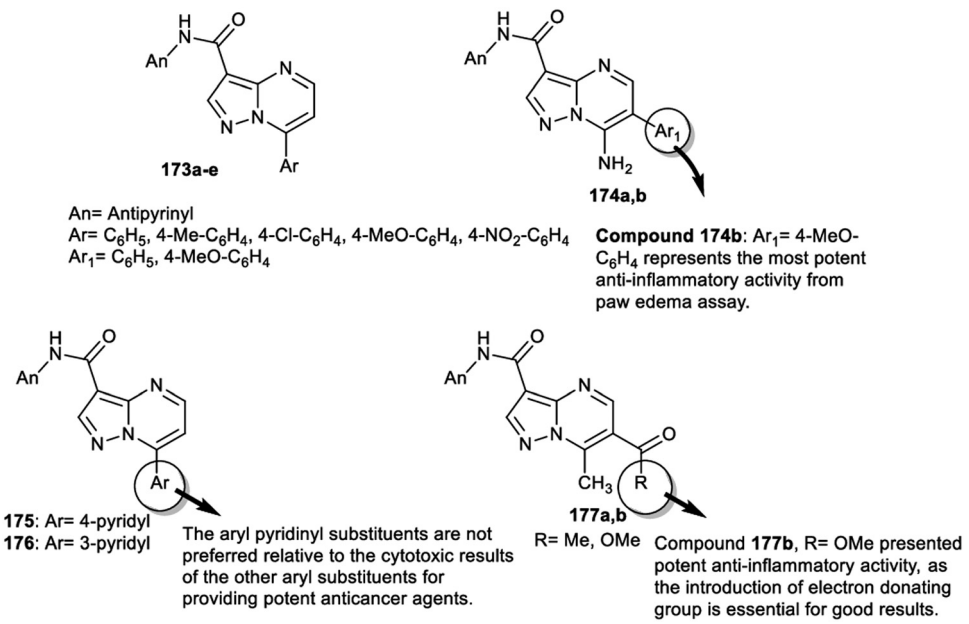


Fig. 21 The SARs of potent anti-cancer and anti-inflammatory agents.

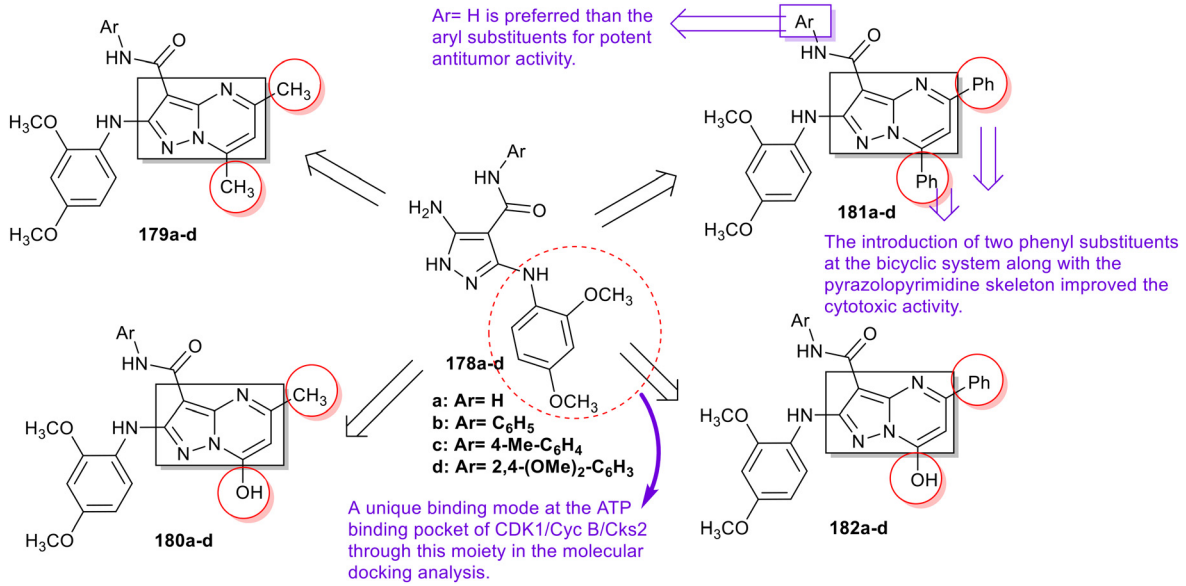


Fig. 22 The structures and SARs of bicyclic pyrazolopyrimidines as potential antitumor agents.

metabolism of MTT solution caused by the action of the enzymes of CHO K1 cells. The order of decreased metabolism of MTT solution was recorded for compounds **173a** (97.10%), **174b** (72.96%), **177a** (72.46%), **177b** (49.24%), **174a** (45.75%), and **173b**. Therefore, compounds **173a**, **173b**, **174a**, **174b**, **177a**, and **177b** have cytotoxic properties on CHO K1 cell lines (Fig. 21).

Four series of pyrazolopyrimidine compounds **179–182a–d** (Fig. 22) were synthesized recently by Elgiushy *et al.*[118] utilizing the reactivity of β-aminoketones **178a–d** towards one-pot reactions with each of acetyl acetone, ethyl acetoacetate, 1,3-diphenylpropane-1,3-dione, and ethyl 3-oxo-3-phenyl propanoate, respectively, under acidic conditions. The results of the antitumor activity indicated that compound **181a** exhibited the most potent activity with 48.5% inhibition against the growth of the 60-NCI cancer cell line relative to the other tested compounds (0.5–10.72%). In addition, the results of *in vitro* MTT assay confirmed that compound **181a** presented very strong potential activity against the HCT-116 colorectal cancer cell line (IC_{50} = 6.28 ± 0.26 μM) and strong activity against normal WI-38 cell line (IC_{50} = 17.7 ± 0.92 μM). The cell cycle over apoptosis detection flow-cytometry and the analysis of gene expression revealed a pro-apoptotic effect of compound **181a** with increased expression of p53, Bax, cytochrome *c*, and caspases along with decreased expression of Bcl-2, thus, the exertion of a pro-apoptotic effect through an intrinsic path. Also, compound **181a** showed good potency for CDK1 inhibition (IC_{50} = 161.2 ± 2.7 nM) relative to roscovitine (IC_{50} = 81.7 ± 1.5 nM).

Sabita *et al.*[119] have reported a multistep synthesis for the construction of isoxazolyl-pyrazolopyrimidines **183a–j** (Fig. 23) and assessed their cytotoxic activity against a diverse tumor cell line by applying the MTT assay. The results indicated potent activities for compounds **183a** (IC_{50} = 0.18 ± 0.069 μm), **183b** (IC_{50} = 0.99 ± 0.052 μm), **183c** (IC_{50} = 1.77 ± 0.96 μm), **183d** (IC_{50} = 2.1 ± 1.25 μm) and **183e** (IC_{50} = 0.08 ± 0.005 μm) against the SiHa cell line. The most potent cytotoxicity was recorded by compounds **183a** (IC_{50} = 0.01 ± 0.0043 μm) and **183e** (IC_{50} = 0.054 ± 0.006 μm) against the A549 cell line. The most potent cytotoxicity was also recorded for compound **183a** (IC_{50} = 0.01 ± 0.0043 and 0.1 ± 0.047 μm) against MCF-7 and Colo-205 cells, respectively. Generally, compounds **183a–e** are the most potent cytotoxic agents against all the assessed cell lines.

A new series of diaryl-pyrazolopyrimidines **184a–i** (Fig. 24) were efficiently synthesized by Ballesteros-Casallas *et al.*[120] and assessed *in vitro* as cytotoxic agents against HCT-116 and HEK 293 cell lines. Compounds **184d** (percentage of cell viability = 26.0 ± 2.3%) and **184h** (percentage of cell viability = 37.0 ± 2.3%) revealed the most potent activities against HCT-116 tumor cells. Otherwise, exceptional compounds **184i** (percentage of cell viability = 44.2 ± 0.4%) and **184b** (percentage of cell viability = 48.0 ± 0.1%) demonstrated good cytotoxic effects compared to the other tested compounds with the most

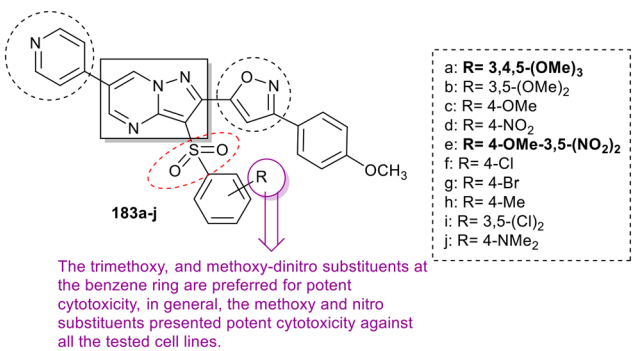


Fig. 23 The structures and SARs of bicyclic-based heterocycles as potential cytotoxic agents.

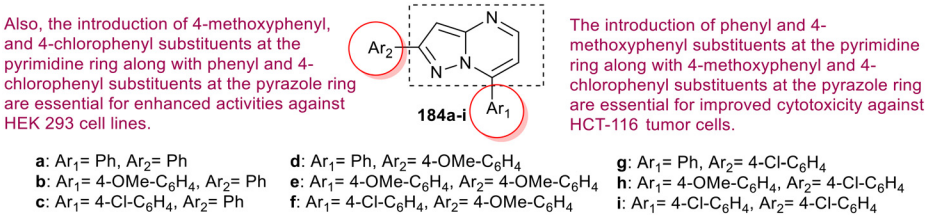


Fig. 24 The structures and SARs of bicyclic heterocycles as cytotoxic agents.

decreased percentages of cell viabilities against HEK 293 cell lines. More recently, Bhogireddy *et al.*[121] have established the anticancer potency of arylpyridinyl-isoxazolyl-pyrazolopyrimidines on PC3 (prostate), DU-145 (prostate), A549 (lung), and MCF-7 (breast) tumor cell lines.

4.5. Anti-inflammatory activity

The anti-inflammatory properties of compounds **49–51**, **54**, and **56** were evaluated using tissue swelling (edema) and nitric oxide assays. The potent anti-inflammatory potency of the tested compound measured the efficiency of the compound to decrease vascular penetrability in the reduction process of the edema. The paw diameter was measured at different intervals of time, ensued a decrease in the paw diameter of some molecules. The results indicated that compounds **49a**, **49b**, **49d**, **49e**, **50b**, **51c**, **54a**, and **54b** revealed good inhibition for paw edema. In addition, bicyclic compounds **6a**, **6d**, and **8b** presented the same potency of paw edema inhibition at 24 h relative to the control group. On the other hand, the nitric oxide assay was used for the evaluation of the anti-inflammatory activity of these compounds as the potent analog can reduce the production of nitric oxide. Compounds **54a**, **54b**, **50c**, **50d**, **50a**, **49e**, **50b**, **51a**, and **51c** revealed reduced concentration in nitric oxide when paw exudates of mice inflamed with FCA were treated with the investigated compound. The nitric oxide concentrations in blood were measured with varying levels, indicating that compound **54c** has the highest level of reduction of nitric oxide concentration, followed by **50c**, **51b**, **56**, and **54a**. In addition, compounds **49d**, **49e**, **50b**, **50d**, and **51a** showed a high level of concentration reduction in NO with equivalent grades; nevertheless, compounds **49a** and **49c** (Fig. 20) are inactive with no reduction in NO concentration.[91]

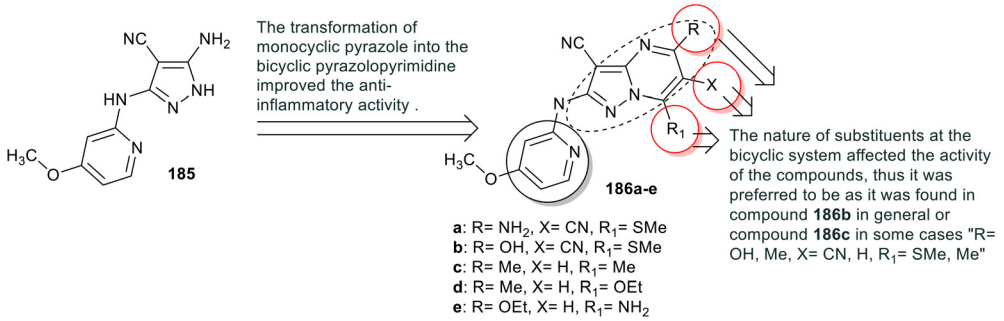
Kaping *et al.*[91] have also reported the evaluation of pyrazolopyrimidines as anti-inflammatory agents by paw diameter as percentage inhibition and nitric oxide assay. The results of the paw diameter assay indicated that 2-aryl-pyrazolopyrimidines **49a**, **49b**, **49d**, **49e**, **50b**, **51c**, **54a**, and **54b** presented inhibition of paw edema. In addition, compounds **50a**, **50d**, and **51b** revealed an equivalent inhibition of paw diameter after 24 h relative to the controlled untreated group. On the other hand, the nitric oxide assay demonstrated that the mice inflamed with FCA revealed a reduction in nitric oxide concentration using compounds **54a**, **54b**, **50c**, **50d**, **50a**, **49e**, **50b**, **51a**, and **51c** (Fig. 20).

The anti-inflammatory activity was evaluated for this series of pyrazolopyrimidines **173–177** (Fig. 21) through paw edema and nitric oxide assays using ibuprofen as a standard. The highest percentage of inhibitions of the edema in the paw diameter of the mice at 24 h were recorded for compounds **174b** (25%) and **177a** (16.57%). However, at 4 h the highest effects were recorded for compounds **174b**, followed by compounds **175**, **177a**, **177b**, **173a**, **173c**, **173e**, and **176**, while the standard ibuprofen revealed the highest percentage of inhibition of up to 66.67% at 4 h. On the other hand, compound **177b** exerted a remarkable reduction in NO concentration in paw exudates, even though compound **173a** revealed an approximate reduction magnitude in blood. Compounds **174b** and **177b** are associated with the maximum aptitude to decrease the noticeable indicators of inflammation, neutrophils and eosinophils.[93]

Abdelgawad *et al.*[122] have reported the synthesis of a series of pyrazolopyrimidines **186a–e** through a simple procedure that involved the utility of the reactive pyrazole precursor **185** in reactions with a variety of unsaturated nitriles or β-diketones. The anti-inflammatory activity was estimated for these series of compounds through *in vivo* and *in vitro* techniques. Alternatively, compound **186b** (Fig. 25) was appraised as the most effective agent against IL-6 (80%) and TNF-α (89%). A potent inhibitory impact was recorded for compound **186c** against COX-2 (IC_{50} = 1.11 μM) along with extreme selectivity against COX-2 (S.I = 8.97) recorded for compound **186b**. The edema assay investigated respectable potency for compounds **186b–e** (46–68%) with the most effective influence for compound **186b** (ED_{50} = 35 mg kg^{-1}). Also, compounds **186b** (IC_{50} = 1 μM) and **186d** (IC_{50} = 1.7 μM) revealed the most effective inhibitions for sPLA2-V. The potency of the compounds against 15-LOX presented a good potential inhibition for compound **186c** (IC_{50} = 5.6 μM) even though it was more potent than nordihydroguaiaretic acid (IC_{50} = 8.5 μM). More recently, Prasada Rao *et al.*[123] have prepared a series of pyrazolo[1,5-*a*]pyrimidine derivatives and assessed their potency against inhibition of TNF-α with remarkable results.

4.6. Analgesic activity

The series of 6-(2-tolyl-hydrazono)-dihydropyrazolo[1,5-*a*]pyrimidinones **187a–d** (Fig. 26) was evaluated as analgesic



Samples	sPLA2-V (IC_{50}, μM	15-LOX (IC_{50}, μM	COX-1 (IC_{50}, μM	COX-2 (IC_{50}, μM	COX-2 (S.I.)
185	3.1 ±0.8	14.2 ±2.8	9.1 ±0.5	4.7 ±1.4	1.93
186a	2.6 ±0.3	27.3 ±4.5	10.2 ±1.6	4.4 ±0.9	2.32
186b	1.0 ±0.8	18.4 ±0.7	12.56 ±2.6	1.4 ±0.5	8.97
186c	3.2 ±1.7	5.6 ±1.2	8.5 ±0.2	1.11 ±0.7	7.66
186d	1.7 ±0.5	8.4 ±1.4	7.9 ±0.9	2.7 ±1.3	3.59
186e	3.2 ±0.4	8.2 ±1.2	8.5 ±0.7	2.2 ±1.3	3.86
Dexamethasone	0.59 ±0.04	-	-	-	-
Celecoxib	-	-	7.34 ±0.2	1.11 ±0.6	6.61
Indomethacin (30 μM)	-	-	0.29 ±0.05	3.65 ±0.4	0.08
Nordihydroguaiaretic acid (16 μM)	-	8.5 ±0.5	-	-	-

Fig. 25 The structures and SARs of potential anti-inflammatory agents.

agents by a hot plate test in SD rats with a concentration of 15 mg kg^{-1} for each dose. Diclofenac was used as a reference standard. After two hours, the molecules revealed potent analgesic activities relative to the results of the reference standard (diclofenac, 84.0 ± 12.5%). Compound **187c** showed analgesic activity that exceeded that of the reference standard. The order of the activities was as follows: compound **187c** (R_1 = CF_3, R_2 = Me, 121.1%) is higher than **187a** (R_1 = R_2 = Me, 96.7%), **187b** (R_1 = Me, R_2 = Ph, 65.9%), and **187d** (R_1 = CF_3, R_2 = Ph, 47.4%). Compound **187b** is the only tested sample that showed slight activity after one hour (43.9%), while the other compounds were inactive at first. Compound **187c** exhibited anti-inflammatory activity by a carrageenan rat paw edema model.[75]

4.7. Antioxidant activity

The antioxidant activity of 6-(2-tolyl-hydrazono)-dihydropyrazolo[1,5-*a*]pyrimidinones **187b** and **187d** (Fig. 26) was evaluated by the ABTS colorimetric assay. The results represented moderate antioxidant activities of both compounds lower than that of the reference standard Trolox (binding activity = 1.0 μM) by five times. Compound **187b** (binding activity = 0.21 ± 0.03 μM) showed better activity relative to compound **187d** (binding activity = 0.19 ± 0.02 μM). Generally, the compounds can trap the free radicals of ABTS.[75]

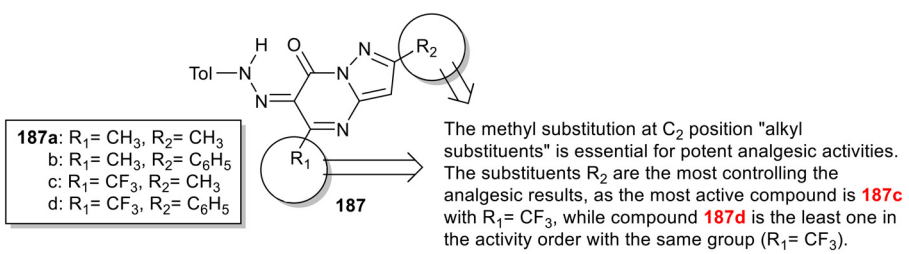
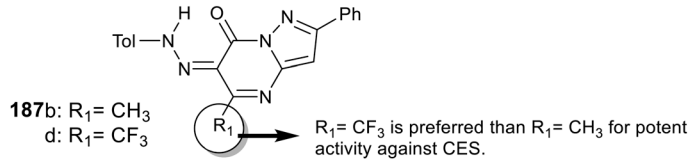


Fig. 26 The SARs of dihydropyrazolo[1,5-*a*]pyrimidinones as potent analgesic and antioxidant agents.



Compounds	Inhibitory activity, IC_{50} ± SEM (μM) at 20 μM		
	AChE	BChE	CES
187a	-	-	-
187b	12.0 ± 2.1%	0.93 ± 0.08%	22.8 ± 2.3%
187c	-	-	-
187d	11.6 ± 1.0%	2.1 ± 0.3%	2.91 ± 0.20%
Tacrine	0.601 ± 0.047%	0.0295 ± 0.0002%	-
Bis(4-nitrophenyl)- phosphate	-	-	1.80 ± 0.11%

Fig. 27 The SARs of pyrazolopyrimidinones as potent carboxylesterase agents.

4.8. Carboxylesterase activity

The carboxylesterase activity was inspected for compounds **187b** and **187d** (Fig. 27) against acetylcholinesterase (AChE) of human erythrocytes, butyrylcholinesterase (BChE) of horse serum, and CES (EC) of pig liver. Tacrine and bis(4-nitrophenyl)phosphate were used as reference standards, in which tacrine is inactive against CES. The results, in general, showed that compound **187b** (R_1 = CH_3) is inactive, while compound **187d** (R_1 = CF_3) inhibited carboxylesterase in a μM concentration range relative to that of bis(4-nitrophenyl)-phosphate. The compounds are inactive against AChE and BChE, with a slight potent activity of compound **187b** against BChE, and compound **187d** is slightly more active than compound **187b** against AChE.[75]

4.9. Antiviral activity

A series of pyrazolopyrimidines **188–197** (Fig. 28) were synthesized in an efficient route to investigate their efficiency as oral respiratory syncytial virus (RSV) fusion inhibitors. Compound **197** was reported as the most effective analog that improves the protein binding of human plasma by adjusting the antiviral potency, permeability, pharmacokinetic properties, and solubility in an aqueous medium that facilitates the formulation of the solution for infants. The key structural changes were the introduction of aminopyrrolidine at the C-5 position of the heterocyclic system. Potential activity was recorded for compound **197** on RSV A and B clinical isolates (n = 75, mean EC_{50} = 0.43 nM) and revealed a dose-dependent (0–30 mg kg^{-1}) antiviral efficacy in a cotton

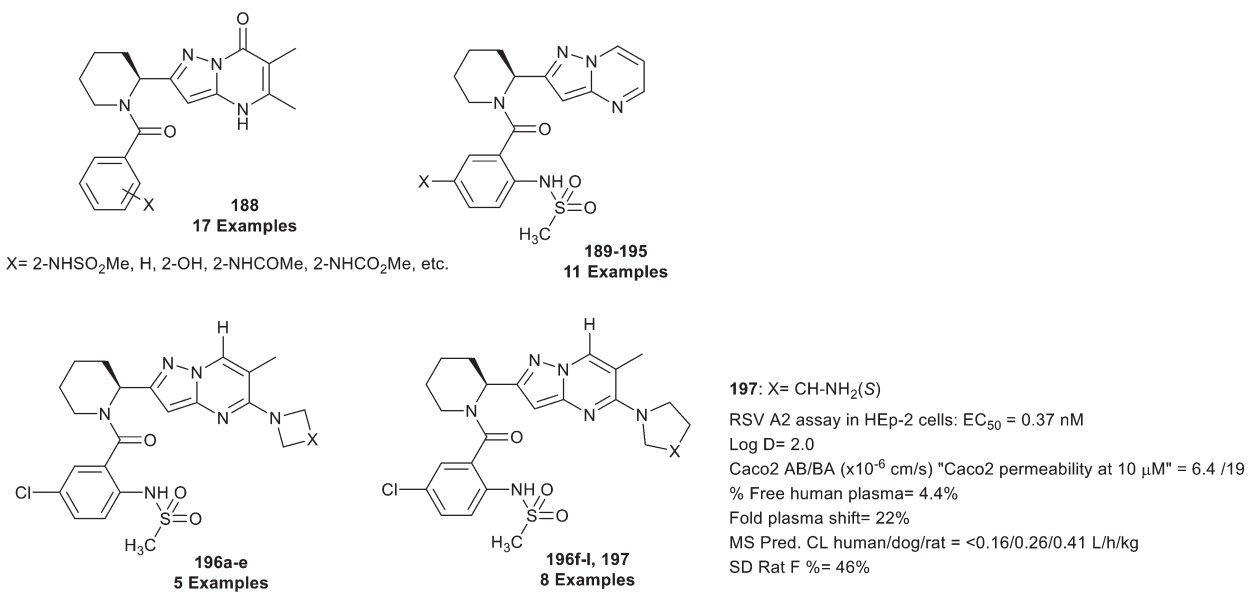


Fig. 28 Structures of pyrazolopyrimidines as oral respiratory syncytial virus fusion inhibitors.

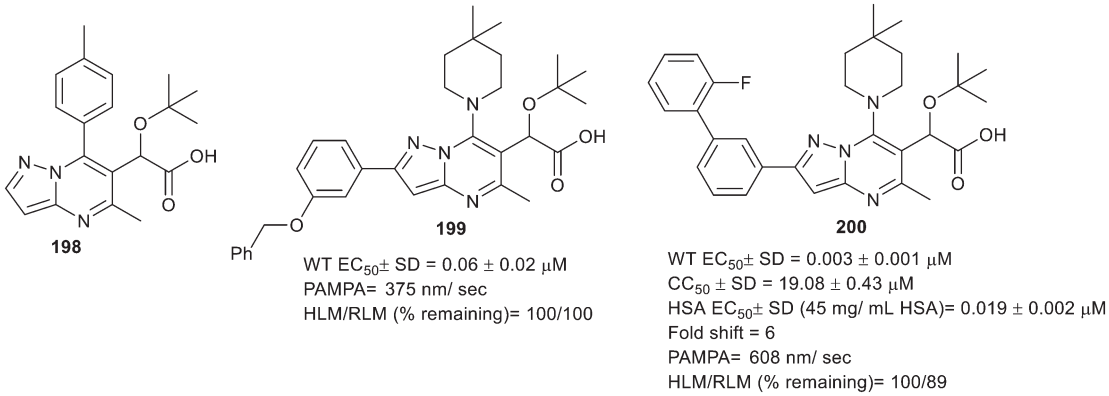


Fig. 29 The structures of potent allosteric inhibitors of HIV-1 integrase.

rat model of RSV infection. Oral treatment with compound **197** was indicated safe in adults and healthy human volunteers tentatively infected with RSV. The results of the high dose verified the potent antiviral effect and reduced disease severity.[124] In another route, other researchers are attentive to studying the antiviral activity of pyrazolopyrimidines.[125–127]

Li *et al.*[128] have reported a combination of two or more drugs for highly active antiretroviral therapy and standard of care for HIV-1 infections through optimization of compound **198** at the 2- and 7-positions to give compounds **199** and **200** (Fig. 29). A series of pyrazolopyrimidines was evaluated as allosteric inhibitors for HIV-1 integrase, which bind to the LEDGF/p75 interaction site and disrupt the structure of the integrase multimer that is essential for HIV-1 maturation. Compounds **199** and **200** exhibited strong allosteric inhibition of HIV-1 integrase with low nanomolar antiviral influence in cell culture and encouraging PK characteristics.

Metwally and Abd-Elmoety[129] have recently utilized the reactivity of pyrazolopyrimidine **201** in the synthesis of arylidenes, hydrazono, and polycyclic systems with the pyrazolopyrimidine motif (Fig. 30). The study was extended to *in vitro* assessment of the antiviral potency of some compounds against COX-B 4, RVF, VSV, and EMCV viruses. The results indicated that compounds **201**, **202c**, **202i**, **203b** and **205** exhibited remarkable potency against the assessed viruses. Additionally, the different assays of antiviral potency demonstrated that compound **202c** exhibited a significant inhibitory impact against hepatitis C virus protease (HCV-NS3) (IC_{50} = 7.33 ± 0.51 μg mL^{-1}) with improved activity compared to compounds **201**, **202i**, **203b**, and **205** and relative to the result of Sovaldi (IC_{50} = 3.20 ± 1.33 μg mL^{-1}).

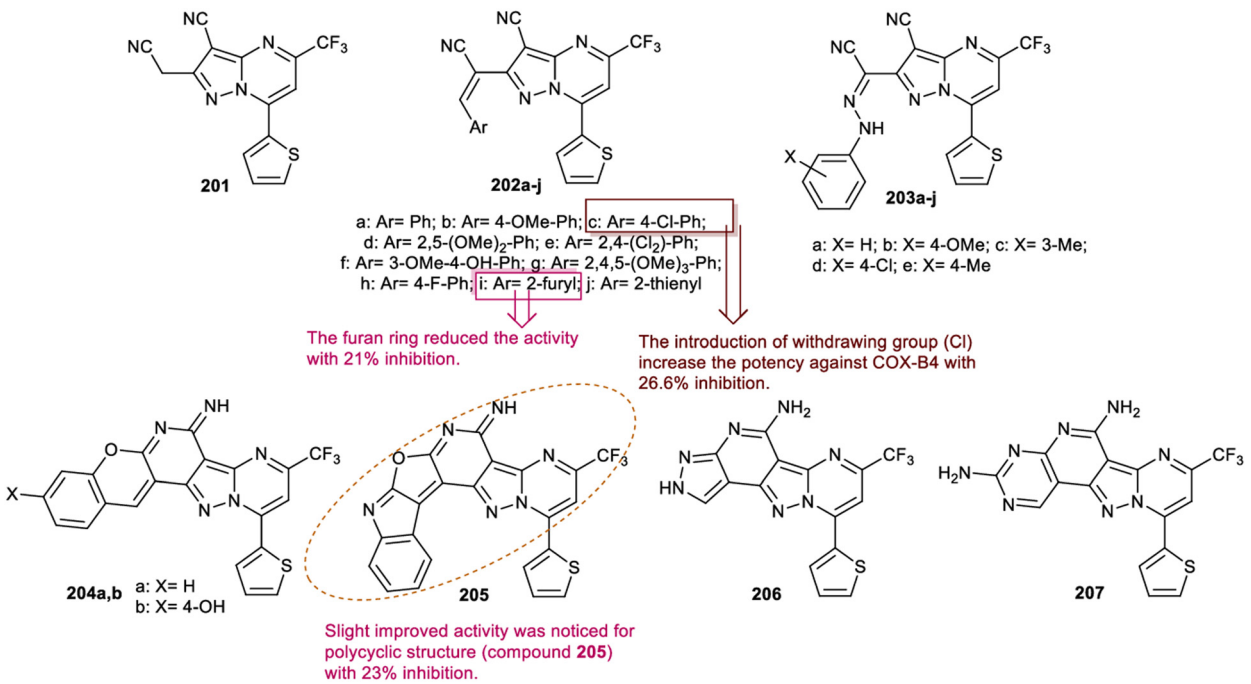


Fig. 30 The SARs of fluorinated bicyclic and polycyclic systems as antiviral agents.

The indirect assay verified improved inhibitory activity with 31.828% inhibition compared to the direct assay (13.8%). The absorption distribution metabolism excretion findings indicated that the adsorption of compound **201** could be achieved through the gastrointestinal area and is superior to that of the other compounds.

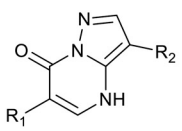
4.10. Anxiolytic effect "antianxiety agents"

The $GABA_A$-R receptor (γ-aminobutyric acid) is an ionotropic considered as the major neurotransmitter inhibitor in the central nervous system with permeable selectivity for chloride ions and lower for bicarbonate ions. The transmission of the GABAergic system plays a critical role in brain activity regulation during the improvement or disruption of its network.[130] The affinity of the $GABA_A$-receptor subtype was assessed *in vitro* for two synthesized series of pyrazolopyrimidinones **19** and **20**. The results showed that compound **20g** has anti-anxiety activity analogous to that of diazepam as a standard reference at a dose of 10–30 mg kg^{-1} (Fig. 31). The activity of compound **20g** was tested as an anxiolytic in mice employing the maze test. In this test, a conditional fear occurs by exposing the mice to open spaces individually to study other materials that do not depend solely on the activity of the mice. The results highlighted the effectiveness of compound **20g** as an anti-anxiety agent in mice. The results also showed the effectiveness of compound **20g** when interacting with subtype receptors of $GABA_A$ (α1, α2, α5) as an attempt to understand and study its biological competence as an anti-anxiety model. Compound **20g** was capable of binding the receptors of the α1-subtype receptor at a nanomolar range (K_i = 183 ± 18 nM) and with no affinity with the α2- and α5-subtype receptors.

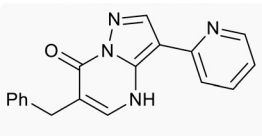
4.11. Translocator protein affinity

Two series of fluorine-based acetamides **60a–c** and **63a–d** were assessed *in vitro* in a binding assay as potent ligands for translocator protein by Tran *et al.*[79] The most evaluated compounds exhibited a potent affinity for translocator protein compared to the results of DPA-714. In particular, compound **60a** exhibited a better affinity for translocator protein with K_i = 0.94 ± 0.045 nM than the reference standard DPA-714 with remarkable lipophilicity for further *in vivo* studies on the brain. The radiolysis of compound **60a** with [^{18}F] yielded the isomeric [^{18}F]**60a** that was applied for a dynamic PET study in a rat LPS-induced neuroinflammation model. The result of the anti-inflammatory study is comparable to that of [^{18}F]DPA-714 with a high accumulation of [^{18}F]**60a** in microglia and an improved TSPO expression location. Immunohistochemical tests of the dissected brains specified that the uptake location of [^{18}F]**60a** in the PET study was reliable, with a positively activated microglia region. This study proved that [^{18}F]**60a** could be engaged as a potential PET tracer for detecting neuro-inflammation and possible diagnosis of other diseases, for example, cancers related to TSPO expression (Fig. 32).

Kwon *et al.*[94] have prepared fluorinated ligands **70a–c** and **71a–c** (Fig. 33) with the pyrazolopyrimidine motif and *in vitro* appraised their proficiency as translocator protein ligands. The compounds demonstrated a nanomolar affinity for translocator protein with high potency. Compound **70a** exhibited the most potent affinity for translocator protein



19a-m: R_1= Ph
20a-m: R_1= CH_2Ph

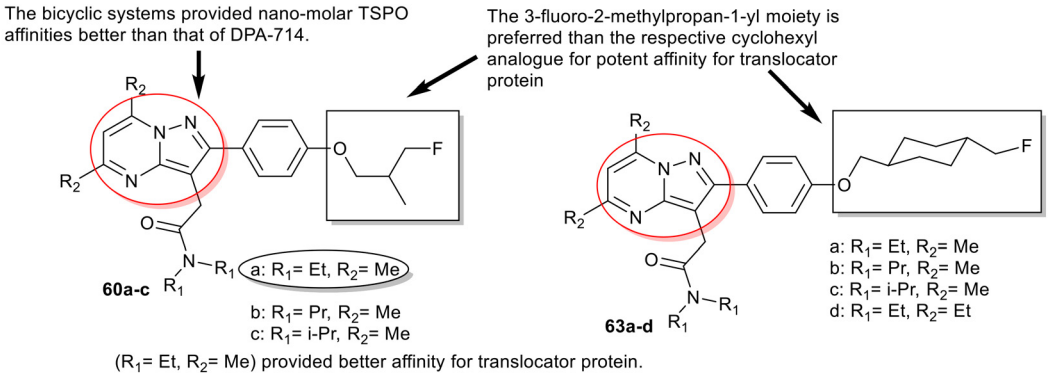


20g
K_i= 29.8 ± 1.6 (nM)

R_2= Ph, 4-F-Ph, 3-Br-Ph, 3-CF_3-Ph, 3-MeO-Ph, 3-Me-Ph, 2-pyridyl, 3-pyridyl, 2-thienyl, 3-thienyl, benzyl, 1-naphthyl, 2-naphthyl

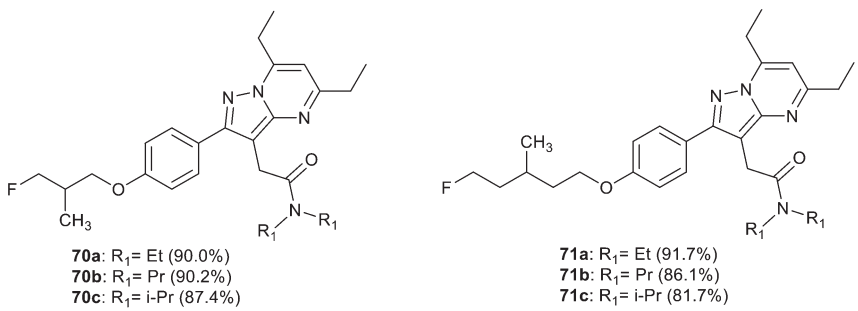
Treatment		% of time in light	Transfer number
Sample	mg/kg p.o.		
CMC	10	118.2 ± 7.8	15.2 ± 3.3
Diazepam	1	171.6 ± 9.3	8.3 ± 2.2
20g	1	113.9 ± 9.4	15.0 ± 3.2
	3	145.2 ± 10.6	21.3 ± 3.5
	10	165.4 ± 7.7	23.9 ± 3.2
	30	164.4 ± 10.3	28.9 ± 4.1
Flumazenil	100	120.08 ± 8.4	12.8 ± 3.5
20g	10		

Fig. 31 The anxiolytic effect of pyridinyl-pyrazolopyrimidinones.



Compounds	K_i (nM ± SD)	Log $P_{7.5}$
DPA-714	3.26 ± 0.390	1.99
60a	0.94 ± 0.045	2.44
60b	1.11 ± 0.074	3.17
60c	1.04 ± 0.065	2.93
60d	3.12 ± 0.065	3.21
63a	1.32 ± 0.067	3.34
63b	1.88 ± 0.072	3.92
63c	1.59 ± 0.093	3.73
63d	2.39 ± 0.054	3.91

Fig. 32 SARs of translocator protein ligands through binding assay.



Compound	R_1	R_2	R_3	K_i (nM ± SD)	Log $P_{7.5}$
70a	Et	$OCH_2CH(CH_3)CH_2F$	Et	3.12 ± 0.61	3.21
70b	Pr	$OCH_2CH(CH_3)CH_2F$	Et	3.86 ± 0.67	3.78
70c	i-Pr	$OCH_2CH(CH_3)CH_2F$	Et	3.90 ± 1.15	3.63
71a	Et	$OCH_2CH_2CH(CH_3)CH_2CH_2F$	Et	3.08 ± 0.03	3.61
71b	Pr	$OCH_2CH_2CH(CH_3)CH_2CH_2F$	Et	4.35 ± 0.57	4.16
71c	i-Pr	$OCH_2CH_2CH(CH_3)CH_2CH_2F$	Et	5.72 ± 1.30	4.0
DPA-714	Et	OCH_2CH_2F	Me	3.26 ± 0.39	1.99

Fig. 33 The structure and biological profiles of translocator protein ligands.

and appropriate lipophilicity. Accordingly, compound **70a** was chosen for *in vivo* studies on the brain, and the location of inflammation was detected. The radio-synthesized fluorinated [^{18}F]**70a** acts as a favorable PET imaging agent for identifying neuro-inflammation and was found to be appropriate for diagnosing cancers with altered translocator

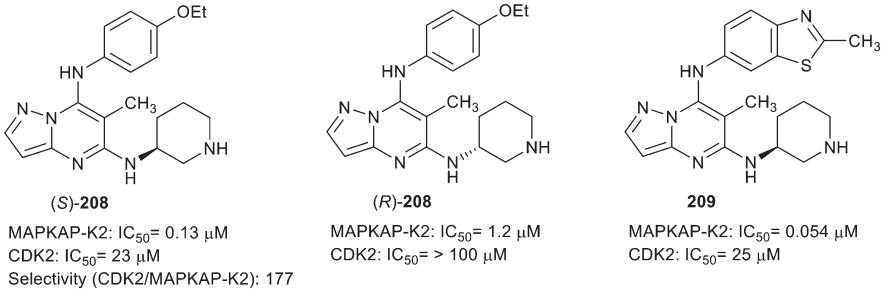


Fig. 34 The structures of proficient protein kinase 2 inhibitors.

protein expression by dynamic positron emission tomography performances.

4.12. Protein kinase inhibitors

Kosugi *et al.*[131] have reported the synthesis of pyrazolopyrimidines **208** and **209** through the reactions of the respective aminopyrazoles with β-ketoesters in sodium ethoxide solution as a basic medium. The compounds revealed potent activities as inhibitors for protein kinase 2 (MAPKAP-K2) along with worthy *in vitro* cellular potency as anti-TNF-α agents and *in vivo* ability in a mouse model of endotoxin shock. The high potency and selectivity of compound **209** led the authors to synthesize compounds with the same skeleton but different aryl amine substituents (*i.e.* compounds **208**) (Fig. 34). The effect of substituents attached to the C6 position on the improved selectivity over CDK2 can be justified. The pyrazolopyrimidine nucleus developed *in vitro* cellular strength in LPS-induced TNF-α secretion cell models and a favorable PK profile. The results revealed exceptional *in vitro* kinase selectivity of (*S*)-**208** in addition to its verified inhibition of HSP27 phosphorylation, a straight substrate of MAPKAP-K2, ensuring that this molecule elicits its influence on TNF-α secretion through inhibition of MAPKAP-K2.

The reaction of 1*H*-pyrazol-5-amine with 1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione yielded compounds **210a–k** after chlorination and nucleophilic substitution with different amines in multi-step synthetic routes. Compounds **211–213** were prepared from pyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid under optimized catalytic conditions. Somatic Janus kinase 2 mutations lead to proliferative tumors, and therefore it was necessary to pay attention to the preparation and discovery of Janus kinase 2 inhibitors to be used in the treatment of these disorders. Pyrazolopyrimidines are considered efficient inhibitors for Janus kinase 2 so the activity of compounds of series **210** against Janus kinase 2 led to high selectivity, in contrast to the other Jak family kinases, and worthy pharmacokinetic characteristics. Compound **210j** (Fig. 35) verified a time-dependent knockdown of pSTAT5 (IC_{50} = 7.4 nM), a downstream target of Jak2 (K_i = 0.1 nM).[132]

Calmodulin-dependent protein kinase II (CaM kinase II or CaMKII) is a serine/threonine-specific protein kinase, which is regulated by the formation of the calcium/calmodulin complex. CAMKII is essential for several signaling cascades, improved activity in numerous cardiac diseases, calcium homeostasis, reuptake in cardiomyocytes (muscle cells containing myofibrils), transportation of chloride in epithelia, selection of positive cytotoxic T cells (a type of white blood cells that inhibit the growth of cancer cells) and activation of CD8 T cells. Aouidate *et al.*[133] reported a series of thirty-six pyrazolopyrimidines as CAMKIIδ kinase inhibitors, in which the compounds are selective inhibitors for CAMKIIδ. The results indicated that the compounds of group 4, *i.e.* compound **214** (R_1 = NM-Me) (Fig. 36), demonstrated potent inhibitory kinase activities. The SARs (Fig. 36) indicated that six regions A–F in the main skeleton of these compounds are responsible for the inhibitory activities. Therefore, the substituents with hydrophilic nature in medium size, electron-donating groups, and hydrogen acceptors at the E-region enhanced the inhibitory activity. Additionally, the electron-deficient groups between the rings C and D improve the inhibitory activity. The research was also extended for the structure characterization by molecular modeling (3D QSAR), docking studies, and *in silico* assessment (ADMET).

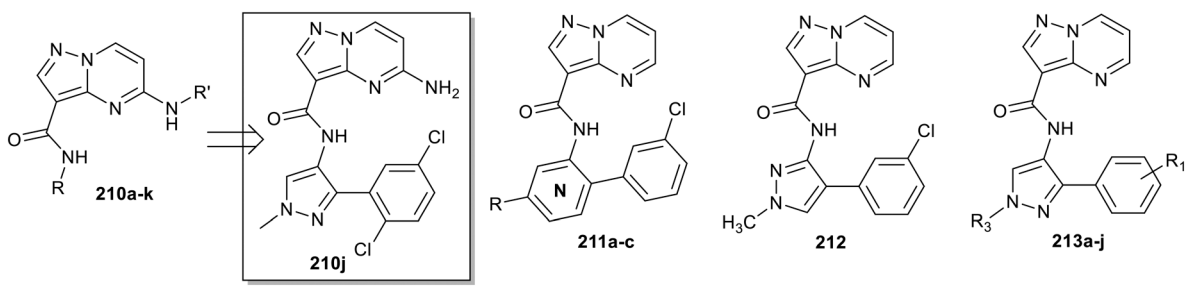


Fig. 35 The structures of potent Janus kinase 2 inhibitors.

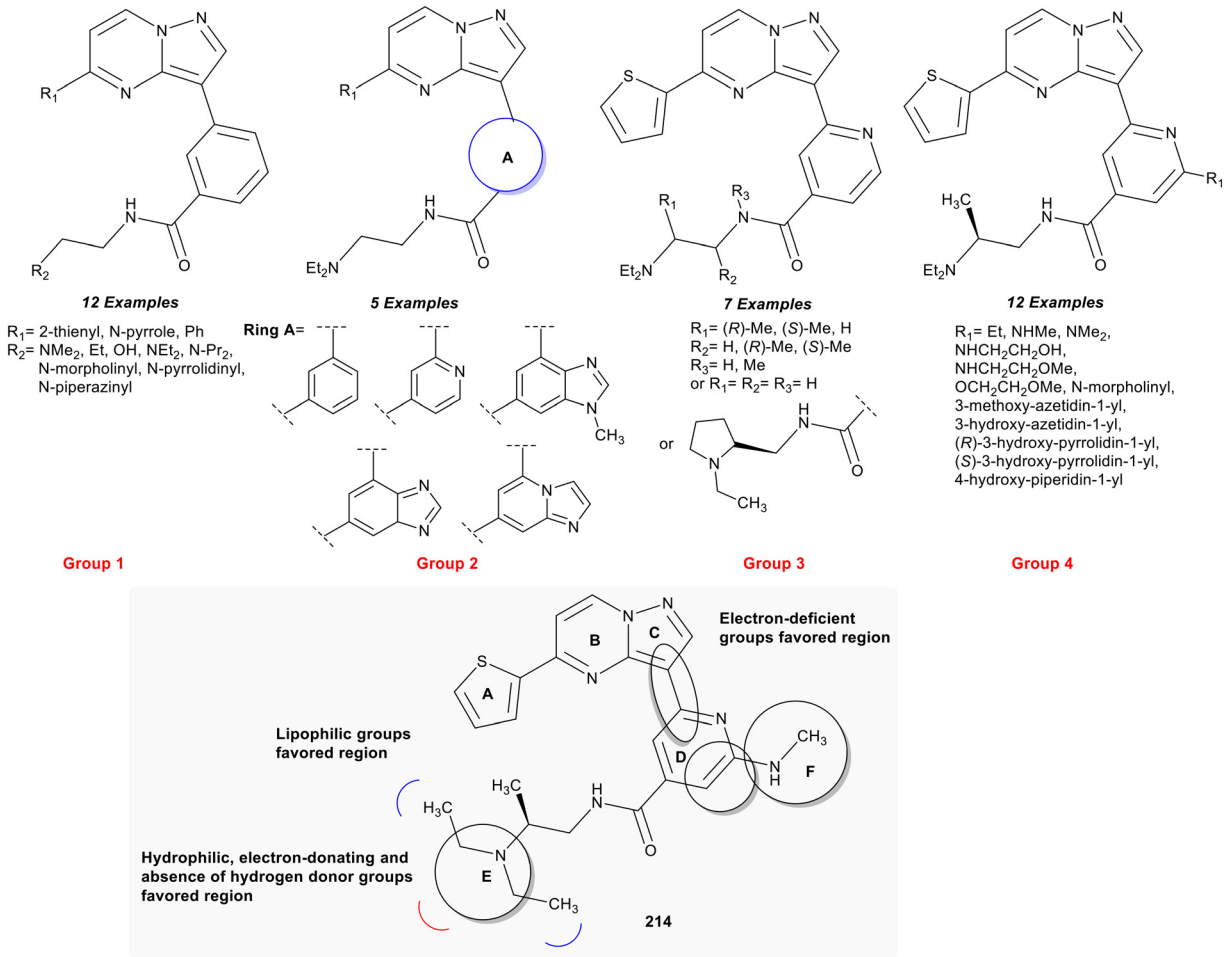
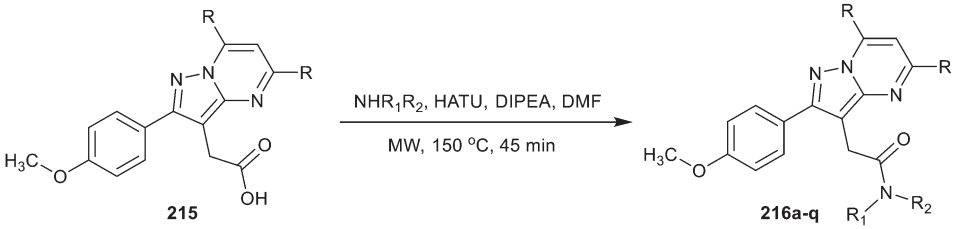


Fig. 36 The structures of pyrazolopyrimidines and SARs of the most active kinase inhibitor **214**.

Therefore, the determination coefficient (R^2) = 0.676 and leave-one-out cross-validation coefficient (Q^2) = 0.956 were calculated.

A series of acetamides **216a–q** were prepared through two step-synthesis from the reaction of the corresponding *tert*-butyl 2-(5-amino-3-(4-methoxyphenyl)-1*H*-pyrazol-4-yl)acetate with heptane-3,5-dione by heating in ethanol followed by basic hydrolysis in an ethanol/sodium hydroxide solution under microwave irradiation conditions. The second step was accomplished by reactions of the formed carboxylic acid with secondary alkyl amines under catalytic conditions to give compounds **216a–q**. Compounds **216a–q** (Fig. 37) were evaluated for their translocator protein affinity, in which the affinity was decreased by the branched alkyl chains and bulky alkyl substituents, *i.e.* compound **216b** revealed results of affinity at 0.18 nM, while compound **216h** revealed a potency at 59.12 nM. The affinity was released by the incorporation of one phenyl substituent with remarkable implications. Phenyl–ethyl substitution is crucial for potent binding translocator protein affinity with picomolar activity (K_i = 0.28 nM). The symmetric benzyl substituents (compound **216n**) decreased the affinity with picomolar activity (K_i = 397.29 nM). The alicyclic substituents (compounds **216o–q**) are not superlative for potent binding affinity. The compounds with long-chain carbons (compounds **216d–f**) have lipophilic characters, while the non-branched or straight chains have moderate to weak lipophilicity. The phenyl substituents revealed a lipophilicity compromise between long alkyl chain and bulky substituents.[134]

Atypical activation of Bruton's tyrosine kinase (BTK) demonstrates a significant role in the pathogenesis of B-cell lymphomas, signifying that inhibition of BTK is beneficial for the treatment of hematological malignancies. In this route, a series of carboxamides **221** were synthesized by bicyclic ring cyclization through reactions of aminopyrazoles **217** with unsaturated ketones accompanied by acidic hydrolysis of the nitrile group, specific reduction of the pyrimidine ring, and finally, chiral separation (Scheme 25). These compounds were evaluated as selective, irreversible, and potent inhibitors for BTK applying *in vitro* potency, selectivity, pharmacokinetic (PK), and *in vivo* pharmacodynamic properties for selected molecules. Compound **221a** (zanubrutinib), in which R_1 = OPh and R_2 = 1-acryloylpiperidin-4-yl, exhibited (1) potent activity on BTK and exceptional selectivity over other TEC, EGFR, and Src family kinases, (2) appropriate ADME, admirable *in vivo* pharmacodynamics in mice and effectiveness in OCI-LY10 xenograft models.[135]



Compound	R	R_1	R_2	Yield %	K_i (nM ± SD)	Log $P_{7.5}$
(DPA-713)	Me	Et	Et	-	4.7	2.40
216a	Et	Me	Me	97	37.59 ± 5.96	2.47
216b	Et	Et	Et	86	0.18	2.84
216c	Et	*n*-Pr	*n*-Pr	82	5.57 ± 2.98	3.35
216d	Et	*n*-Bu	*n*-Bu	82	7.34 ± 0.89	3.81
216e	Et	n-pentyl	*n*-pentyl	80	2.17 ± 0.74	4.29
216f	Et	*n*-hexyl	*n*-hexyl	79	18.57 ± 10.27	4.78
216g	Et	Me	*t*-Bu	64	94.79 ± 43.05	3.13
216h	Et	Et	*t*-Bu	70	59.12 ± 16.12	3.32
216i	Et	*iso*-Pr	*iso*-Pr	67	20.03 ± 1.56	3.26
216j	Et	*iso*-Bu	*iso*-Bu	85	13.25 ± 1.76	3.77
216k	Et	2-Me-Pr	2-Me-Pr	76	14.40 ± 8.07	3.75
216l	Et	Me	Ph	51	6.44 ± 1.44	3.11
216m	Et	Et	Ph	49	0.28 ± 0.14	3.27
216n	Et	-CH_2-Ph	-CH_2-Ph	76	397.29 ± 69.39	3.73
216o	Et	Azetidin-1-yl		96	87.75 ± 37.96	2.58
216p	Et	Pyrrolidin-1-yl		83	127.20 ± 13.13	2.72
216q	Et	Piperidin-1-yl		84	49.22 ± 9.24	2.96

Fig. 37 Translocator protein affinity of pyrazolopyrimidinyl-acetamides.

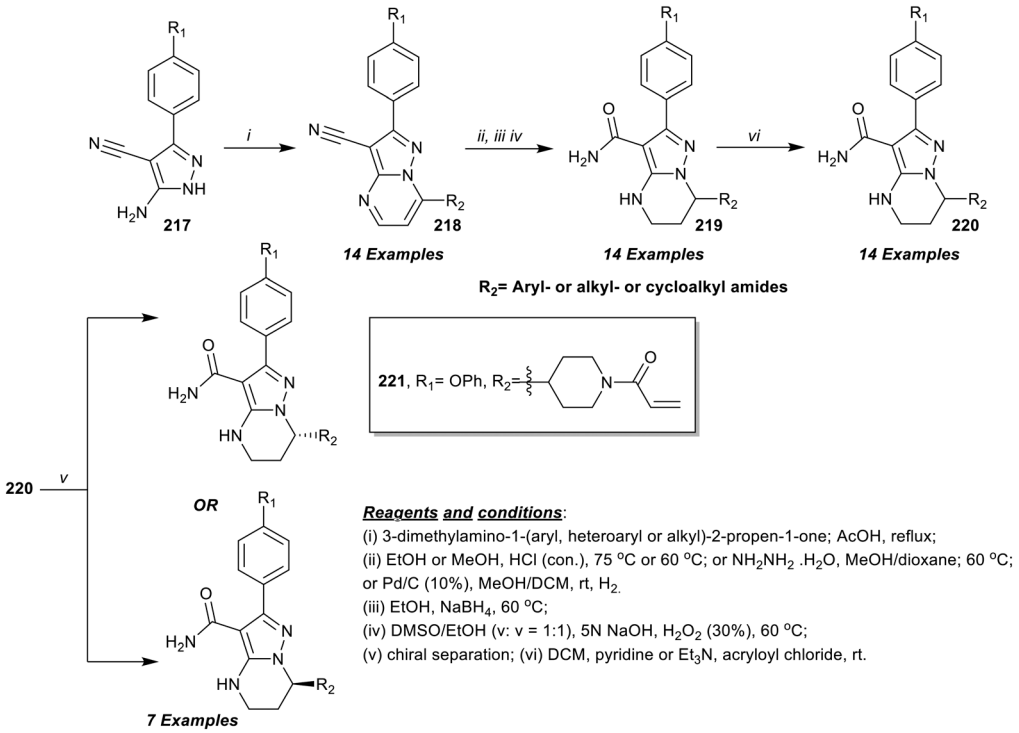
Gopalsamy *et al.*[136] identified the basic hydrolysis of the ester group of compound **222** with lithium hydroxide to yield the desired acid, which was coupled with amines under catalytic conditions at room temperature to give the diamide analogs **223**, **224**, **225a–d**, and **226a–d**. B-Raf kinase exhibited a precarious protagonist in the Raf–MEK–ERK signaling pathway and its inhibitors might be applied in the treatment of melanomas, colorectal cancer, and other Ras-related human cancers. A series of pyrazolopyrimidines substituted with ethyl carboxylate or their analogous amides **223**, **224**, **225a–d**, and **226a–d** were investigated as B-Raf inhibitors by HTS assay (Fig. 38).[136] Recently, Kurz *et al.*[137] have synthesized pyrazolopyrimidine-based macrocyclic skeletons and investigated their selectivity of inhibition for serine/threonine kinase 17A.

4.13. PDE10A inhibitor

The main role of the PDE10A gene is to encode phosphodiesterase-enriched cyclic nucleotides of medium striatum spiny neurons in the brain.[138] PDEs are hydrolases that degrade intracellular signaling compounds vital to cellular functions such as cAMP and cGMP. Koizumi *et al.*[139] have indicated the potency of quinoxalinyl-pyrrolidinyl-based pyrazolopyrimidine **227** as an inhibitor of PDE10A with high selectivity. Applying the rat CAR test, the compound was effective with positive symptoms of schizophrenia. Compound **244** exhibited promising efficiency in rat-dependent anticipation response tests and appropriate pharmacokinetic assets in rats, specifically extraordinary brain penetration, as presented in Fig. 39.

4.14. Phosphodiesterase 10A inhibitors

Raheem *et al.*[140] have described the synthesis of pyrazolyl-pyridinyl-pyrazolopyrimidine (**PyP-1**) (**229**) through two synthetic routes from 5,7-dichloropyrazolo[1,5-*a*]pyrimidine (**228**) (Scheme 26). The compound efficiently acted as an inhibitor of PDE10A with subnanomolar potency (PDE10A K_i = 0.23 nM) and exceptional pharmacokinetic (PK) and physicochemical characteristics. The compound's performance as an antipsychotic agent for improved cognition was examined by pharmacodynamic (PD) assays depending on the dose efficiency. The PET enzyme occupancy revealed that **PyP-1 229** was shown



Scheme 25 Synthesis of carboxamides.

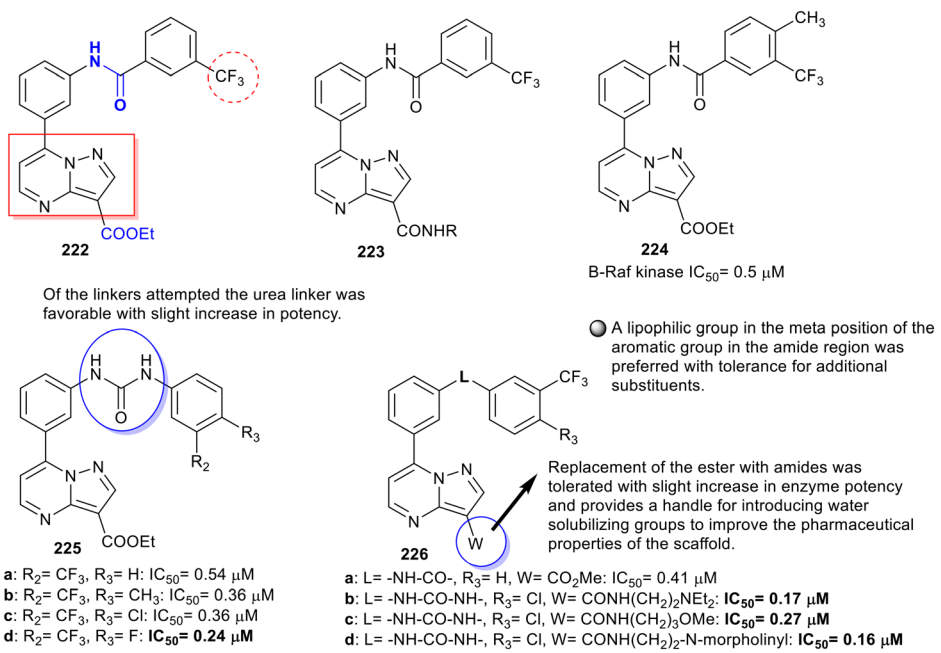
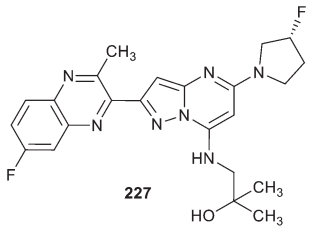


Fig. 38 The SARs of potent B-Raf inhibitors.

in vivo preclinical target assignation concerning [^{11}C]MK-8193, a novel PDE10A positron emission tomography (PET) tracer.

4.15. Phosphodiesterase (PDE4) inhibitors

Phosphodiesterase (PDE-4) inhibitors are identified as a worthy target for the treatment of asthma and COPD. The reactions of 3-aryl-1*H*-pyrazol-5-amines with 3-(3,4-dialkoxyphenyl)-3-oxopropanal or its enaminone analog in acetic acid at room temperature produced the individual 2-arylpyrazolopyrimidines **230** in 60–90% yield. The results of the biological evaluation of this series of compounds as PDE-4 inhibitors revealed that high potency was noticed for the vehicle group relative to the control group. Compound **230p**



human PDE10A IC_{50}= 0.062 nM
bovine PDE10A IC_{50}= 0.090 nM
ODEs selectivity (vs. IC_{50}) > 1000 fold
rat PK; BA^a= 43.7%, $Kp_{,u}{}^a$= 0.50
$t_{1/2}{}^b$= 3.09 h, $Vd_{55}{}^b$= 526 mL/h/kg
$CL_{tot}{}^b$= 2307 mL/kg
rat CAR ID_{50}= 0.32 mg/kg, p.o.

Fig. 39 The structure of potent PDE10A inhibitor.

reduced the eosinophil peroxidase (EPO) activity by about 50% of the vehicle group. The results indicated that compound **230p** (Fig. 40) exhibited a protective effect on the ovalbumin-induced asthma animal model and its valuable effect outcomes comparatively from the suppression of eosinophil infiltration.[141]

4.16. MALT1 protease inhibitors

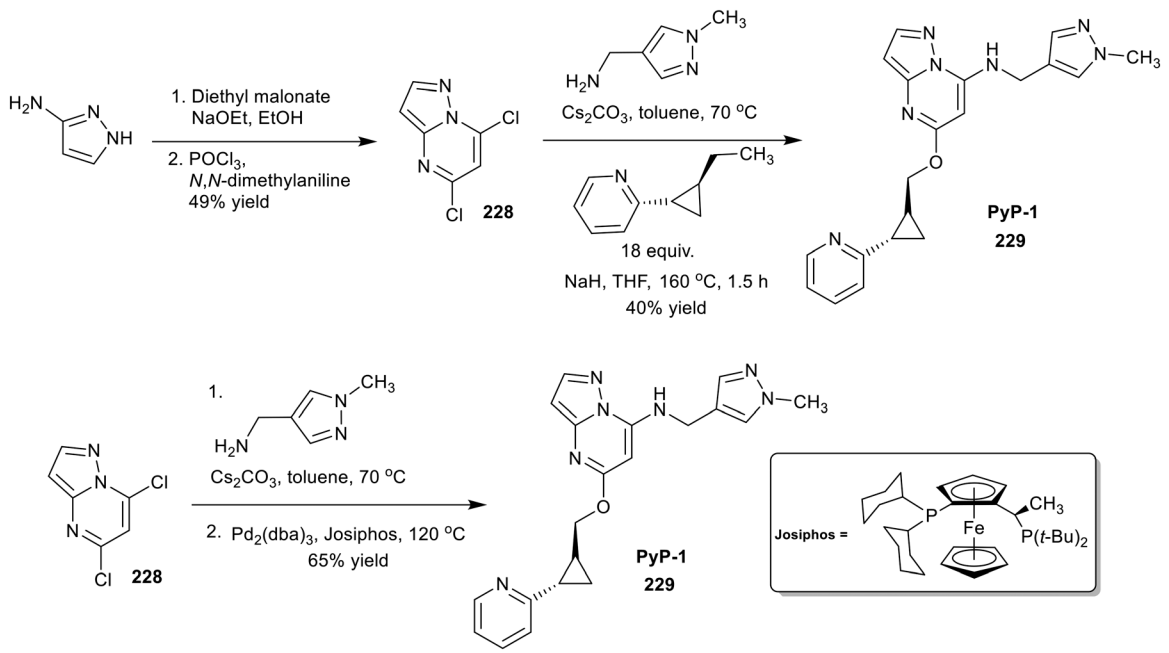
Quancard *et al.*[142] have reported a facile synthesis of a series of pyrazolopyrimidines **231–238** with a substituted urea side chain and optimized the *in vivo* potency of these compounds as selective inhibitors for allosteric MALT1. The high dose was not effective at first, causing diffuse large B cell lymphoma (DLBCL) in a xenograft model along with shortened half-life and suboptimal strength in whole blood. The amended *in vivo* potency of these compounds was achieved by masking one hydrogen bond donor of the central urea moiety *via* intramolecular interaction. Tumor regression was recorded for this compound in a CARD11 mutant ABC-DLBCL lymphoma xenograft model. The subsequent compound **233** (Fig. 41) presented reduced *in vitro* metabolism along with reduced clearance and increased half-life in rats.

4.17. Potassium modulator channel

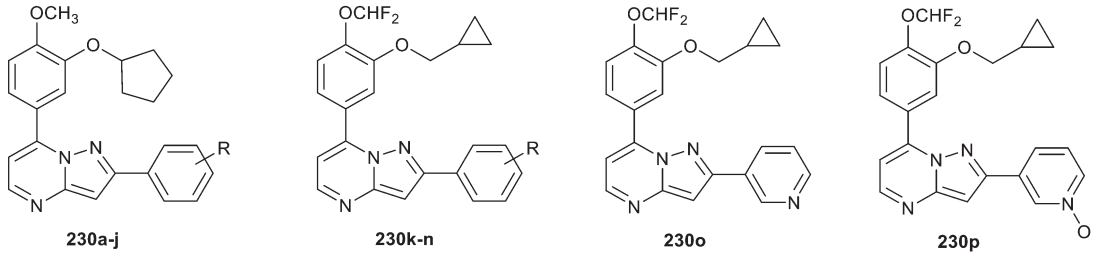
Osuma *et al.*[143] have reported a strategy for the synthesis of carboxamides **241–243** (Fig. 42) and assessed these compounds as KCNQ channel modulators. Hence, KCNQ has the potency for treating CNS disorders comprising neuropathic pain. Potassium channels consist of membrane-bound proteins, which regulated the flow of potassium ions through the cell membrane. The research has discovered the affinity of the investigated compounds for potassium channels KCNQ2/3. The results of the pyrazolopyrimidine compound investigation revealed potent efficiency in a capsaicin-induced acute, secondary mechanical allodynia model and excellent pharmacokinetic properties relative to the results of the standard retigabine.

4.18. Glutamate receptors

The presynaptic group II metabotropic glutamate receptors (mGlu2 and mGlu3) are mostly expressed in the CNS and signify vital therapeutic targets for several CNS disorders, *i.e.*, anxiety, depression, schizophrenia, pain, addiction, Alzheimer's disease and Parkinson's disease. Two series of carboxamides **244** and **245** were synthesized through multi-



Scheme 26 Synthesis of **PyP-1**.



Compound	R	IC_{50} (nM)	cAMP	Compound	R	IC_{50} (nM)	cAMP
230a	H	70	--	**230i**	3-OMe	40	20.1 [a]
230b	2-Br	140	--	**230j**	2,5-Cl_2	30	10.4 [a]
230c	2-OMe	70	--	**230k**	3-Br	13	57.1 [b]
230d	4-Cl	90	--	**230l**	3-I	6	19.8 [b]
230e	4-Br	130	--	**230m**	3-OMe	30	44.4 [b]
230f	4-OMe	60	--	**230n**	3-CO_2Me	37	--
230g	3-Cl	20	19.7 [a]	**230o**	pyridine	27	66.9
230h	3-Br	30	11.4 [a]	**230p**	N-oxide	42	1.61 [c]

[a] % cAMP level relative to control at 20 μM. [b] % cAMP level relative to control at 10 μM. [c] EC_{50} of **247p** for cAMP accumulation.

Fig. 40 Phosphodiesterase (PDE-4) inhibitors against U937 cells.

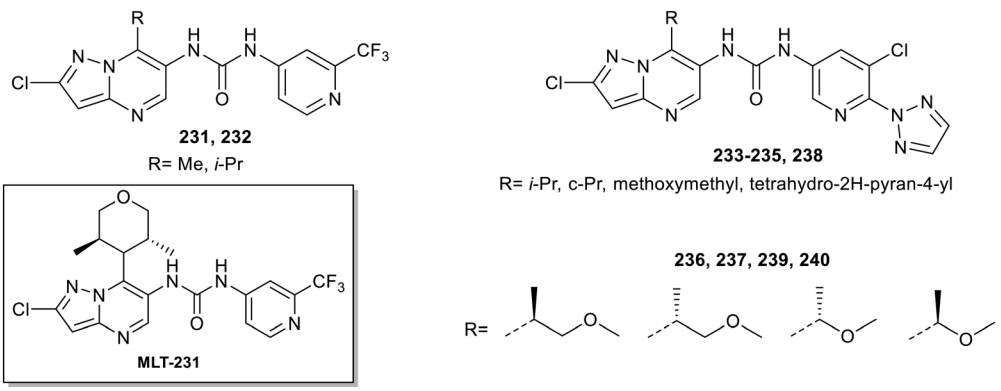
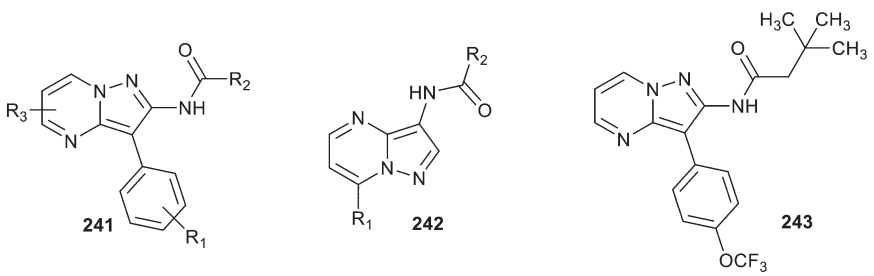


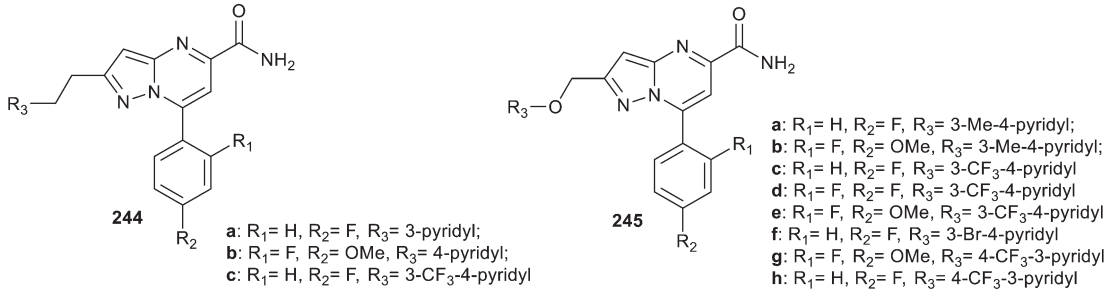
Fig. 41 The structures of model examples of pyrazolopyrimidines as MALT1 protease inhibitors.



The Pharmacokinetics of Compound 243: CLp (iv, 1 mpk) = 0.8 L/h/kg, $t_{1/2}$ (iv, 1 mpk) = 1.9 h, F (po, 1 mpk) = 56%, AUC (po, 1 mpk)= 706 ng-h/ml, Brain/Plasma=3.8

Fig. 42 Pyrazolopyrimidines with minimal abuse potential for pain treatment.

step reactions following bicyclic ring construction, nucleophilic substitutions, oxidation, and amidation reactions. These compounds are highly CNS penetrant, with respectable functional potency and selectivity against the



Compound	mGlu2, IC_{50} (nM) (pIC_{50} ±SEM)	% Glu (Max± SEM)	Compound	mGlu2, IC_{50} (nM) (pIC_{50} ±SEM)	% Glu (Max± SEM)
244a	1738 (5.76 ± 0.05)	2.9±0.1	**245d**	102 (6.99 ± 0.09)	1.5±0.3
244b	1047 (5.98 ± 0.05)	2.6±0.30	**245e**	148 (6.83 ± 0.03)	2.1±2.0
244c	87 (7.06 ± 0.06)	2.3±0.2	**245f**	224 (6.65 ± 0.11)	19.7±10.8
244a	> 10000 (< 5)	--	**245g**	347 (6.46 ± 0.09)	4.2±7.4
244b	288 (6.54 ± 0.03)	35.4±2.0	**245h**	> 10000 (< 5)	--
244c	794 (6.10 ± 0.07)	2.4±0.4			

Fig. 43 The sample examples of pyrazolopyrimidines as glutamate receptors.

other seven mGlu receptor subtypes. Prominently, an analog within this series was the first mGlu2 NAM to indicate an attractive rat *in vivo* PK profile (low clearance and moderate half-life). Excitingly, these new chemotypes did not permanently reveal an IVIVC, making reliance on *in vitro* DMPK assays theoretically problematic. While the ideal *in vivo* mGlu2 NAM did not result from this scaffold-hopping and optimization campaign, advances in CNS penetration coupled with rat PK were appreciated (Fig. 43).[144]

In previous research, pyrazolopyrimidines demonstrated remarkable biological characteristics, for instance, as alpha 1 selective ligands,[145] potent active calcium-sensing receptor antagonists,[81] selective CB2 cannabinoid receptor inverse agonists,[146] corticotropin-releasing factor receptor antagonists,[147] selective inhibitors for a tyrosine kinase,[148] B-Raf,[149] Pim-1,[26a] mitochondrial branched-chain aminotransferase,[150] CNS penetrant,[151] metalloproteinase (MMP-13),[152] IKur,[153] acyl-CoA: diacylglycerol acyltransferase,[154] allosteric modulators for the hydroxycarboxylic acid receptor (GPR109A),[155] and were used for the treatment of Gaucher disease.[156]

5. Conclusion

The current review provided an insight into the medicinal chemistry of heterocycles with a pyrazolopyrimidine core. The cyclization of bicyclic systems was accomplished by cyclocondensation of aminopyrazole derivatives with active reagents such as α,β-unsaturated ketones, β-diketones, enaminones, β-ketoesters, 1,5-diketones, β-ketoaldehydes, enaminonitriles, unsaturated esters, or β-ketonitriles under various conditions of the reactions. The reactivity of substituents linked to the ring carbon enables the synthesis of aryl ethers, diamides by reactions with amines or acid chlorides, synthesis of unsaturated ketones, CH arylation, and synthesis of polycyclic systems and binary heterocycles. In this survey, we highlighted the recent research that reported the various biological profiles of the heterocycles with a pyrazolo[1,5-*a*]pyrimidine nucleus, including the activity of the compounds such as antimicrobial, antimalarial, antioxidant, *etc.* The structure–activity relationships studied the effects of the chemical composition, and the effect of substituents on the results of the biological activities were also studied. The nature of the substituents varies; they are either alkyl groups, aryls, amides, esters, sulfonyl groups, arylamines, aryl amides, or heterocyclic systems. The pyrazolo[1,5-*a*]pyrimidine skeleton was also found as the basic nucleus of heterocycles such as tricyclic and polycyclic systems. Among this class of compounds are those that showed distinct biological activity. Methyl or hydrophilic groups are preferred for the potent efficiency of the tested compounds, while the aryl groups reduce the solubility of these compounds in general, which led to a decrease in their biological potency as expected. The heterocycles with a pyrazolo[1,5-*a*]pyrimidine skeleton presented notable, varied, and privileged biological attitudes, which provide the possibility for applying these molecules on a large scale for drug design.

6. Future perspective

Recently, some studies depended on the synthesis of compounds similar to some drugs in chemical structure, with the substitution of the substituted groups with others similar to them in cyclic properties. The biological activities of the pyrazolo[3,4-*d*]pyrimidine isomers such as pyrazolo[4,3-*d*]pyrimidines have been assessed in several areas, which can also be evaluated for this class of compounds on the same biological pattern. Through the future view of these

heterocyclic compounds, we found that this class of compounds is rich in its distinct biological activity, which can be applied in drug discovery, design, and development through clinical applications.

7. Abbreviations

DPPM	4,7-Dihydropyrazolo[1,5-*a*]pyrimidine
AMP phosphodiesterase	Adenosine monophosphate phosphodiesterase
KDR kinase	Kinase insert domain receptor
COX-2	Cyclooxygenase-2
HIV-1	Human immunodeficiency virus 1
DPA	2-(2-(4-Alkoxyphenyl)-5, 7-dimethylpyrazolo[1,5-*a*]pyrimidin-3-yl)acetamide
Pim-1	Proto-oncogene serine/threonine-protein kinase
CK2	Casein kinase 2
HCV	Hepatitis C virus
CRF	Chronic renal failure
Serotonin 5-HT	Serotonin or 5-hydroxytryptamine
GABAA	Gamma-aminobutyric acid type A receptors
GABA	Neurotransmitter gamma-aminobutyric acid
PET	Positron emission tomography (a functional imaging technique that uses radioactive substances known as radiotracers to visualize and measure changes in metabolic processes; PET scan is a type of test that may be used in cancer treatment)
DDR1	Discoidin domain receptor 1
CAN	Ceric ammonium nitrate
TBAF or *n*-Bu_4NF	Tetra-*n*-butylammonium fluoride
HATU	1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluoro-phosphate, hexafluorophosphate azabenzotriazole tetramethyl uronium
DIBAL-H	Diisobutylaluminium hydride
DavePhos	2-Dicyclohexylphosphino-2′-(*N*,*N*-dimethylamino)biphenyl
DES	Deep eutectic solvent
gla. AcOH	Glacial acetic acid
MIC	Minimum inhibitory concentration
SAR	Structure–activity relationship
$POCl_3$	Phosphoryl chloride
DHODH	Dihydroorotate dehydrogenase
BGM	Buffalo green monkey cells
A549	Lung cancer
MDA-MB-231	Breast cancer
DU-145	Prostate cancer
HEK293	Human embryonic kidney cells
SiHa	Hypertriploid human cell line
HeLa	Human cervical cancer cell line
IC_{50}	Half-maximal inhibitory concentration
EDCI	1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (activates the carboxylic group for coupling reactions with amines)
HOBt	Hydroxybenzotriazole (enables the formation of amides)
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT assay is a colorimetric assay for assessing cell metabolic activity)
MCF-7	Human breast cancer
PC3	Human prostate cancer
PCa	Prostate cancer
AR	Androgen receptor
HepG-2	Human hepatocellular carcinoma
HCT-116	Colorectal adenocarcinoma
Colo-205	Colon cancer
CHO K1	Mammalian cell line used for mass production of therapeutic proteins
NO	Nitric oxide
SD	Sprague Dawley rat
AChE	Acetylcholinesterase
BChE	Butyrylcholinesterase
RSV	Respiratory syncytial virus
EC_{50}	Half maximal effective concentration
TSPO	Translocator protein
[^{18}F]DPA-714	*N*,*N*-Diethyl-2-(2-(4-(2[^{18}F]-fluoroethoxy)phenyl)-5,7-dimethylpyrazolo[1,5-*a*]pyrimidin-3-yl)-acetamide
TNF alpha	Tumour necrosis factor alpha
QSAR	Quantitative structure–activity relationship
PDE	Phosphodiesterases
cAMP	Cyclic adenosine monophosphate
cGMP	Cyclic guanosine monophosphate
EPO	Eosinophil peroxidase (an enzyme found within the eosinophil granulocytes, innate immune cells of humans and mammals)
CNS	Central nervous system
COX-B 4	Coxsackie virus B 4
RVF	Rift Valley fever
VSV	Vesicular stomatitis virus
EMCV	Encephalomyocarditis virus

Conflicts of interest

There is no conflict of interest to declare.

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