# ChemInform Abstract: Recent Advanced in Bioactive Systems Containing Pyrazole Fused with a Five Membered Heterocycle

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Review article

# Recent advanced in bioactive systems containing pyrazole fused with a five membered heterocycle

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ABSTRACT

In this review we report the recent advances in bioactive system containing pyrazole fused with a five membered heterocycle, covering the time span of the last decade. All of them are represented around the common structure of the pyrazole ring fused with another five membered heterocycle containing the nitrogen, sulfur and oxygen atoms in all their possible combinations. The classification we have used is based in terms of the therapeutic area providing, when possible, some general conclusions on the targets and mechanisms of action as well as the structure-activity relationships of the molecules.



## 1. Introduction

The presence of a heterocyclic ring, functioning as core of drug molecules, is an event that occurs commonly in many natural and synthetic drugs. In particular, five and six membered heterocyclic systems are scaffolds of many efficacious drugs. Among these, pyrazole is a versatile aromatic five-membered ring endowed with diverse biological activity on humans such as antimicrobial [1], anti-inflammatory [2], anticancer [3], antiviral [4], anticonvulsant and antidepressant [5].

Moreover, the fusion of two different heterocyclic rings can result in novel scaffold, and heterocycles condensed to pyrazole ring are an important source of bioactive molecules. Although literature is full of reviews focused on the importance of pyrazoles as source of biologically active compounds [6–8], very little is reported on bioactive fused pyrazoles [9]. Taking into account that the synthesis and the biological activity of pyrazoles and fused pyrazoles have been for quite long time under study in our laboratory, with the present review we want to provide an overview of diverse pharmacological activities of condensed heterocyclic systems containing the pyrazole ring, covering the time span of the last decade. In particular, we want to review systems containing pyrazole fused with another five membered heterocycle having the nitrogen, sulfur, oxygen and selenium atoms in all their possible combinations (Fig. 1) by pointing our attention on bicyclic and fully aromatic systems and discarding all the others.

In the present review, for each class, our main objectives was to describe the pharmacological activities and, wherever possible, the targets and mechanisms of action as well as the structure-activity relationships of the molecules. Systems containing pyrazole fused with a five membered heterocycle have been extensively studied for many applications including antitumor agents, antimicrobial agents, antiviral agents, anti-inflammatory agents, agent acting on CNS disorders, androgen receptor modulators, inhibitors of $K_{ir}3.1$ and $K_{ir}3.4$ potassium channels subunits subtypes, and skeletal muscle contractility modulators.

Despite the large number of compounds reported in this review, the structures can be clustered into ten categories (Fig. 2): thieno[3,2-c]pyrazoles **1**, thieno[2,3-c]pyrazoles **2**, furo[3,2-c]pyrazoles **3**, pyrrolo[3,2-c]pyrazole **4**, pyrazolo[3,4-d]thiazoles **5**, imidazo[4,5-c]pyrazoles **6**, pyrazolo[3,4-d][1,2,3]triazoles **7**, pyrrolo[2,3-c]pyrazole **8**, pyrazolo[3,4-c]pyrazole **9**, furo[2,3-c]pyrazoles **10**, pyrrolo[3,4-c]pyrazoles **11** and selenolo[3,2-c]pyrazole **12**.

## 2. Antitumor activity

Literature survey revealed that the most representative biological activity among pyrazoles fused with a five membered

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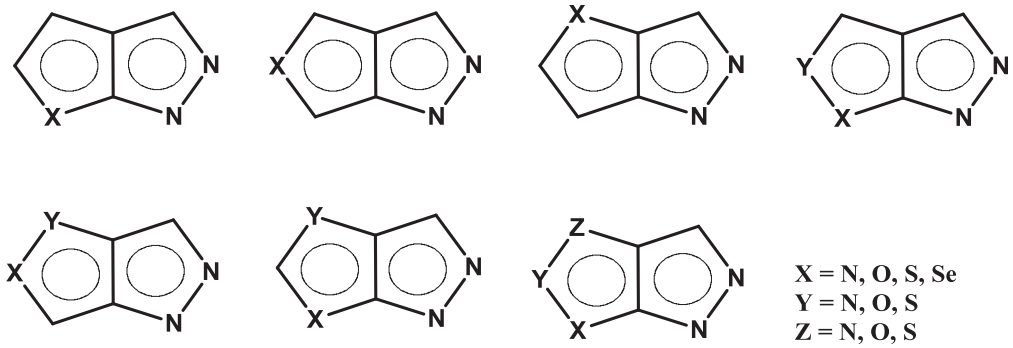



**Fig. 1.** Possible combinations of pyrazole fused with a five membered heterocycle.

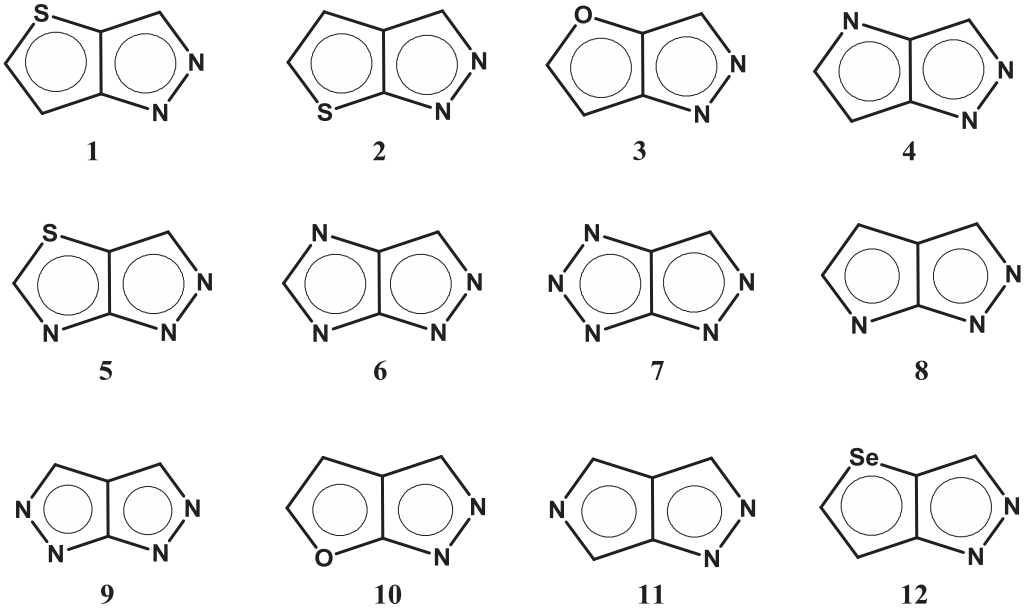



**Fig. 2.** General structures pyrazoles fused with a five membered heterocycle reported in this review.

heterocycle is the antitumor activity. We report about thirty references on this topic which is a very high number compared to all the other activities described here. All the structures can be clustered into six categories (Fig. 2): thieno[3,2-c]pyrazoles **1**, thieno [2,3-c]pyrazoles **2**, furo[3,2-c]pyrazoles **3**, pyrrolo[3,2-c]pyrazole **4**, pyrazolo[3,4-d]thiazoles **5**, imidazo[4,5-c]pyrazoles **6,** pyrazolo [3,4-d][1,2,3]triazoles **7** and selenolo[3,2-c]pyrazole **12**.

### *2.1. Pyrazoles condensed with a five membered heterocycle having one heteroatom*

Fancelli et al. [10] reported that 1*H*-thieno[2,3-c]pyrazoles **13**, wherein R is a substituted aryl or heteroaryl group, $R_1$ and $R_2$ are a hydrogen atom, a straight or branched C1–C3 and taken together a cycloalkyl group, have showed a high affinity for the ATP pocket of ABL tyrosine kinase, displaying a significant inhibitory activity towards BCR-ABL inhibitor-resistant T315I mutants and, in particular, towards Imatinib-resistant T315I ABL mutants.

The inhibiting activity of compounds was determined by a gel-kinase assay. Moreover, inhibiting activity in cellular systems was also reported. The evaluation of compounds ability of binding to ATP pocket of T315I ABL mutant afforded Kd values in the range 0.0041–0.047 μM. Compounds of type **13** are useful for the manufacture of a medicament to treat a BCR-ABL inhibitor-resistant T315I ABL-mediated disease.

The same authors [11] reported the synthesis, their use as therapeutic agents and the preparation of pharmaceutical compositions containing them, of a series of analogs of derivatives **13**. The 1*H*-thieno[2,3-c]pyrazoles **14**, wherein $R_1$ and $R_2$ are the same of derivatives **13**, $R_4$ is a hydrogen or halogen atom, A is an aryl or heteroaryl ring, Z is direct bond or C═O or −C(=O)NH- and $R_5$ is a hydrogen or an organic group, are endowed with a very high Aurora-2 kinase inhibiting activity (Fig. 3).

Since Aurora kinases, in particular Aurora-2, are implicated in the growth of cancer cells, these compounds are useful in the treatment of a variety of cancers. Aurora kinases are also implicated in the regulation of cellular proliferation, therefore these thienopyrazole derivatives are also useful in the treatment of non-malignant diseases such as benign prostrate hyperplasia, familial adenomatosis, neurofibromatosis, psoriasis, vascular smooth cell proliferation associated with atherosclerosis, pulmonary fibrosis and post-surgical stenosis.

Another series of thieno[3,2-c]pyrazole derivatives **15a**–**h** with a potent activity against kinases were described by Bindi et al. [12]. All the synthesized compounds have been shown to inhibit both enzyme (Aurora-A) and cell proliferation (HCT-116). A preliminary

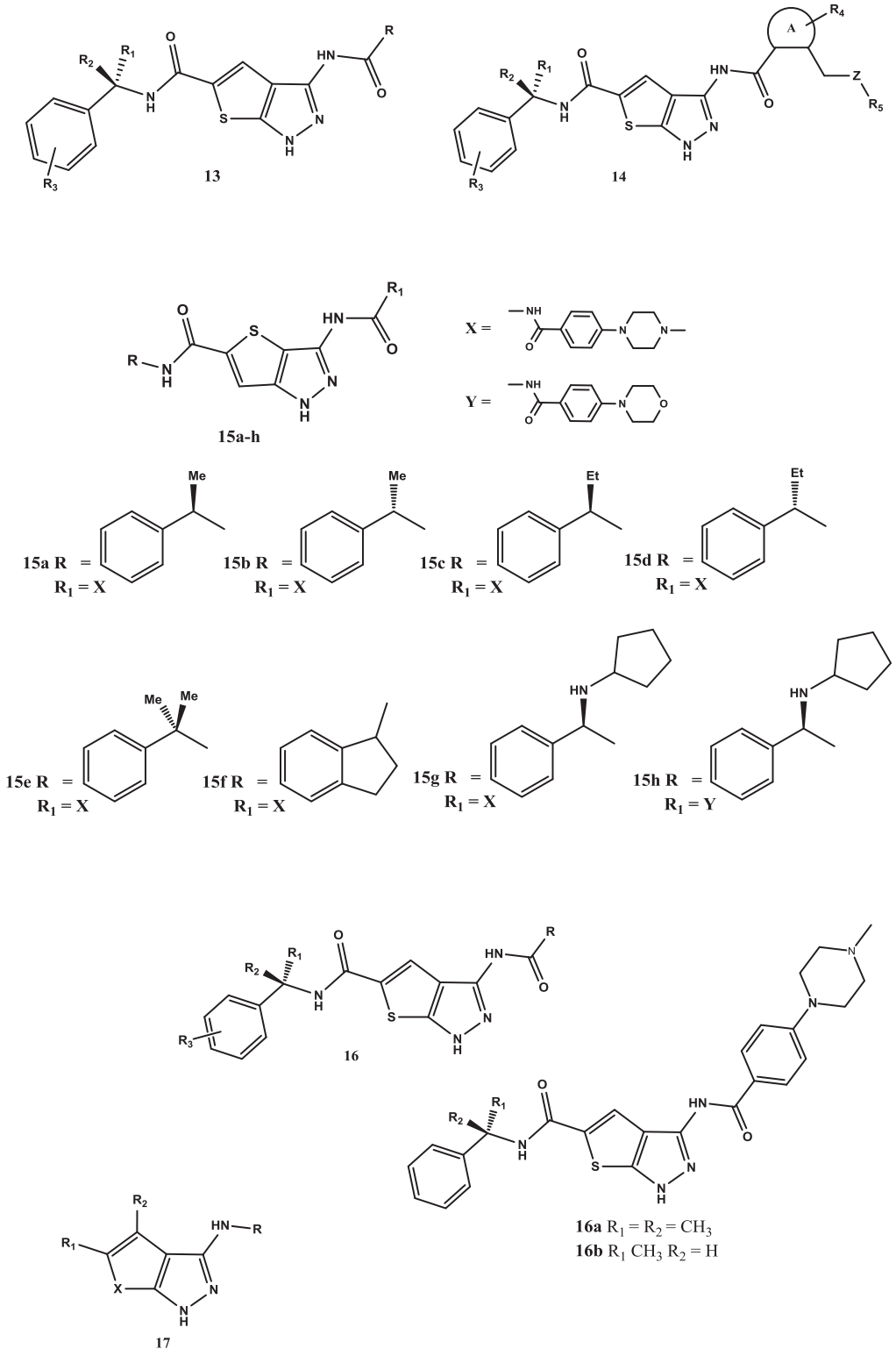



**Fig. 3.** Structures of compounds **13**–**17**.

SAR carried on seven compounds showed how the best substituent in the position 5 is a benzylamide, preferably substituted by small alkyl group in alpha position; the Pro-R configuration was preferred (**15a** vs **15b** and **15c** vs **15d**) as well as the gem di-methyl substitution (**15e**) whilst cyclic analogs are less active (**15f**) (Fig. 3).

Considering that the most potent compounds in this set showed a limited solubility, through docking studies on compound **15c** it was possible to identify the site for attaching solubilizing groups. In particular, the ethyl group sits in a relatively wide cavity so solubilizing groups, taking advantage of this cavity, could substitute the ethyl group. On the basis of this consideration was synthesized 3-(4-methylpiperazin-4-yl-benzoylamino)-1*H*-thieno[3,2-*c*]

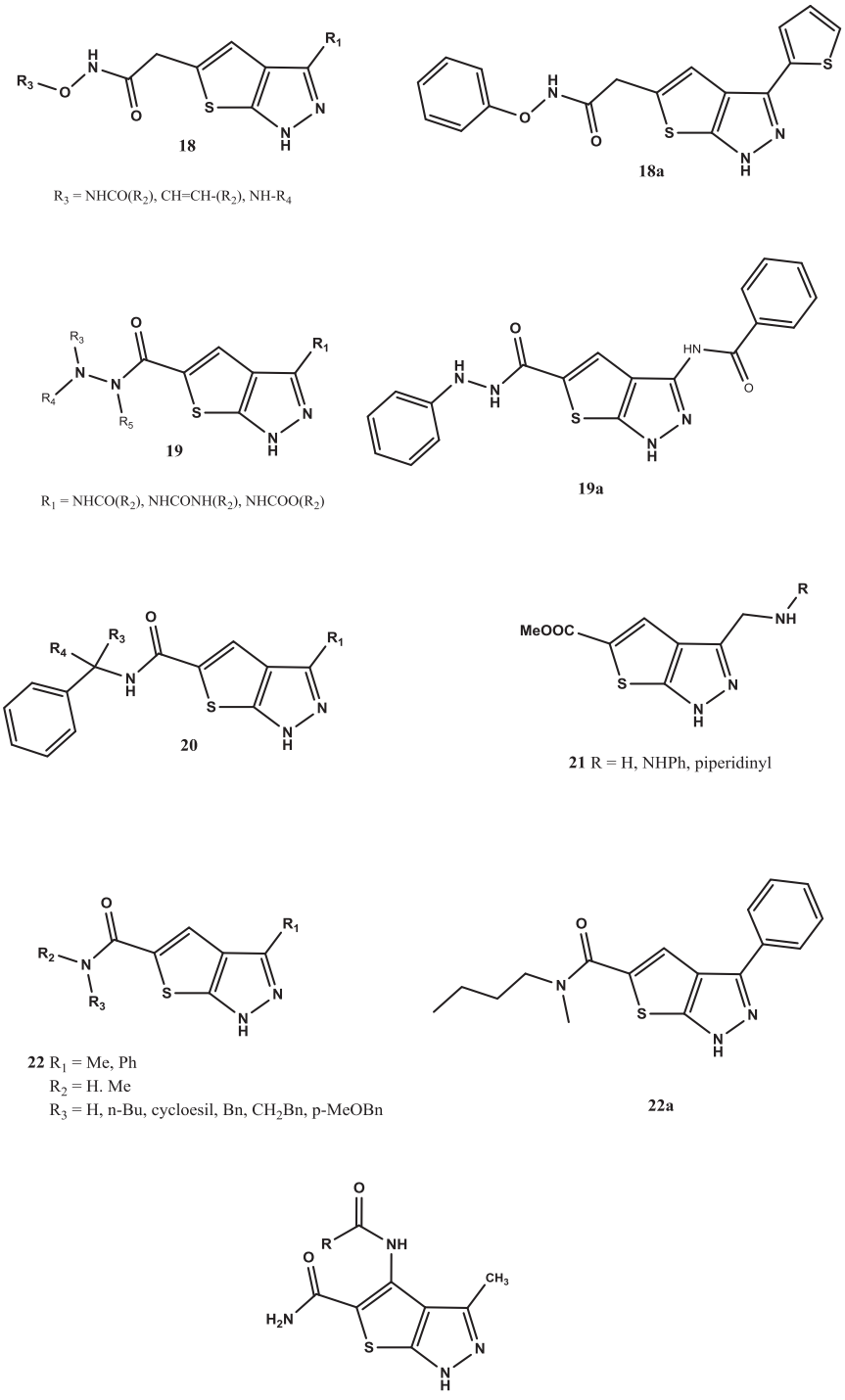



**Fig. 4.** Structures of compounds **18**–**23**.

pyrazole-5-carboxylic acid ((*S*)-1-phenyl-2-pyrrolidin-1-yl-ethyl)-amide **15g** which showed a high increase of solubility. The analog 3-(4-Morpholin-4-yl-benzoylamino)-1*H*-thieno[3,2-*c*]pyrazole-5-carboxylic acid ((*S*)-1-phenyl-2-pyrrolidin-1-yl-ethyl)-amide **15h** resulted to have similar solubility when compared to **15g** resulting more active in the preliminary *in vitro* tests. It was screened against a panel of 40 kinases and, among several families of Tyr and Ser-Thr kinases, it showed the best activity against Aurora kinase with $IC_{50}$ values ranging from 18 nM against Aurora A to 62 nM against Aurora C (Fig. 3).

Other examples of thieno-Pyrazoles useful in the treatment of cancer and associated disorder are compounds **16** [13]. They act by inhibiting the activity of protein Kinases and in particular the Aurora 2-Kinasi. All tested compounds showed $IC_{50}$ values for Aurora-2 inhibition below 20 μM. Compounds **16a** and **16b** were the most active in both assays showing an $IC_{50}$ of 1 nM as Aurora-2 Kinase inhibitors and an $IC_{50}$ of 2 nM and 5 nM respectively as antiproliferative agents (Fig. 3).

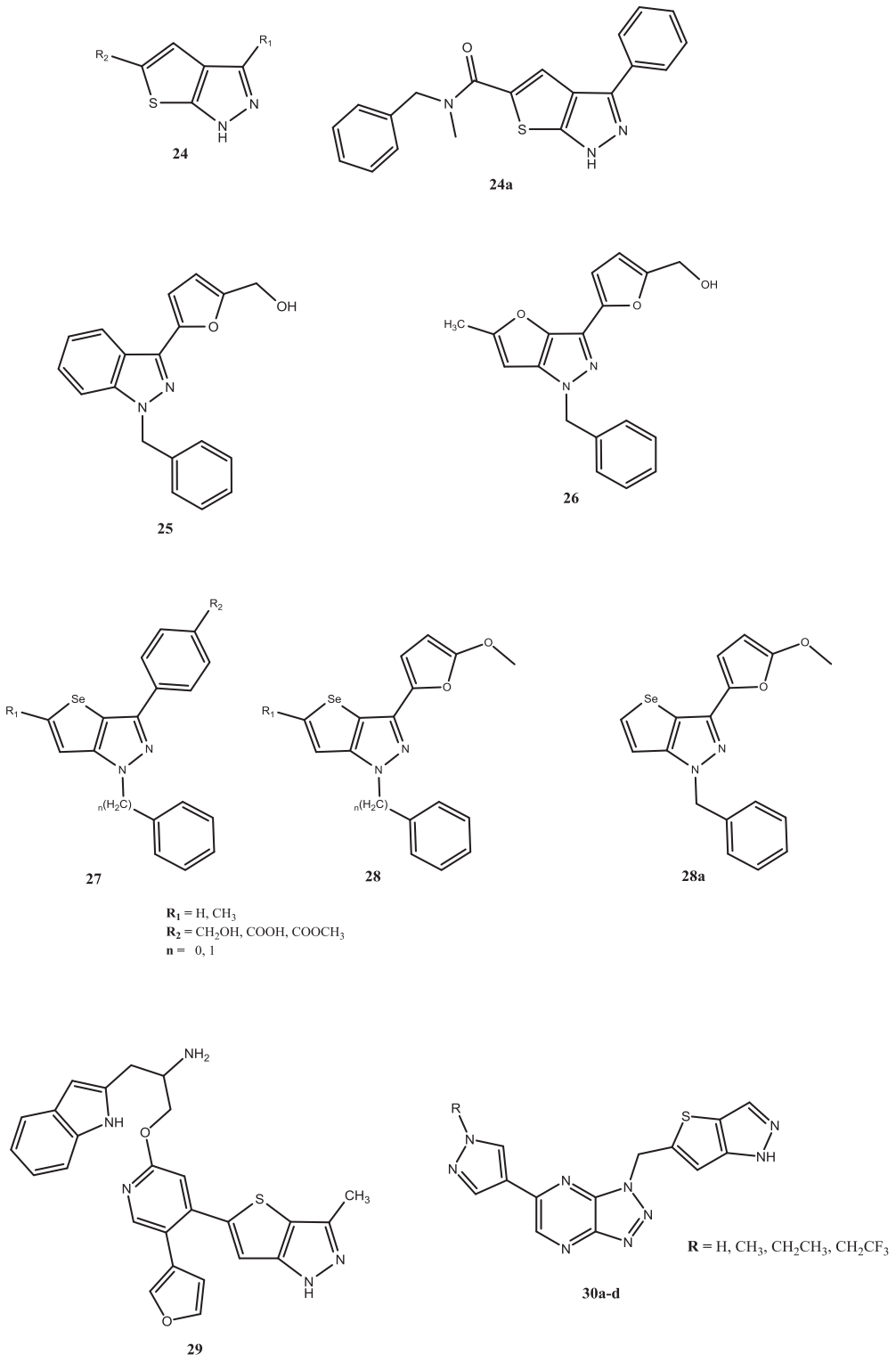



**Fig. 5.** Structures of compounds **24**–**30**.

Tonani et al. [14] presented a patent on bycyclopyrazoles **17** that are useful in therapy as protein kinase inhibitors, modulators of deregulated protein kinase activity and proliferation inhibitors of tumor cells. A very large number of derivatives have been considered but, the preferred substitutions for the best activity are those wherein X is a sulfur atoms, R is −CONHR′, $R_1$ is −CONHR′ or −CONHR″, wherein each of R'and R″, being different or the same, is selected from hydrogen or an optional substituted straight or branched C1−C6 alkyl group, aryl or aryl-C1-C6-alkyl group and R2 is hydrogen (Fig. 3).

Several other compounds containing the thieno[2,3]pyrazole scaffold were described by different authors as inhibitors of Aurora 1, Aurora 2, CDK2 and Tie2 [15–17]. In particular, Carry et al. [15] synthesized a series of thienopyrazole derivatives of type **18**,

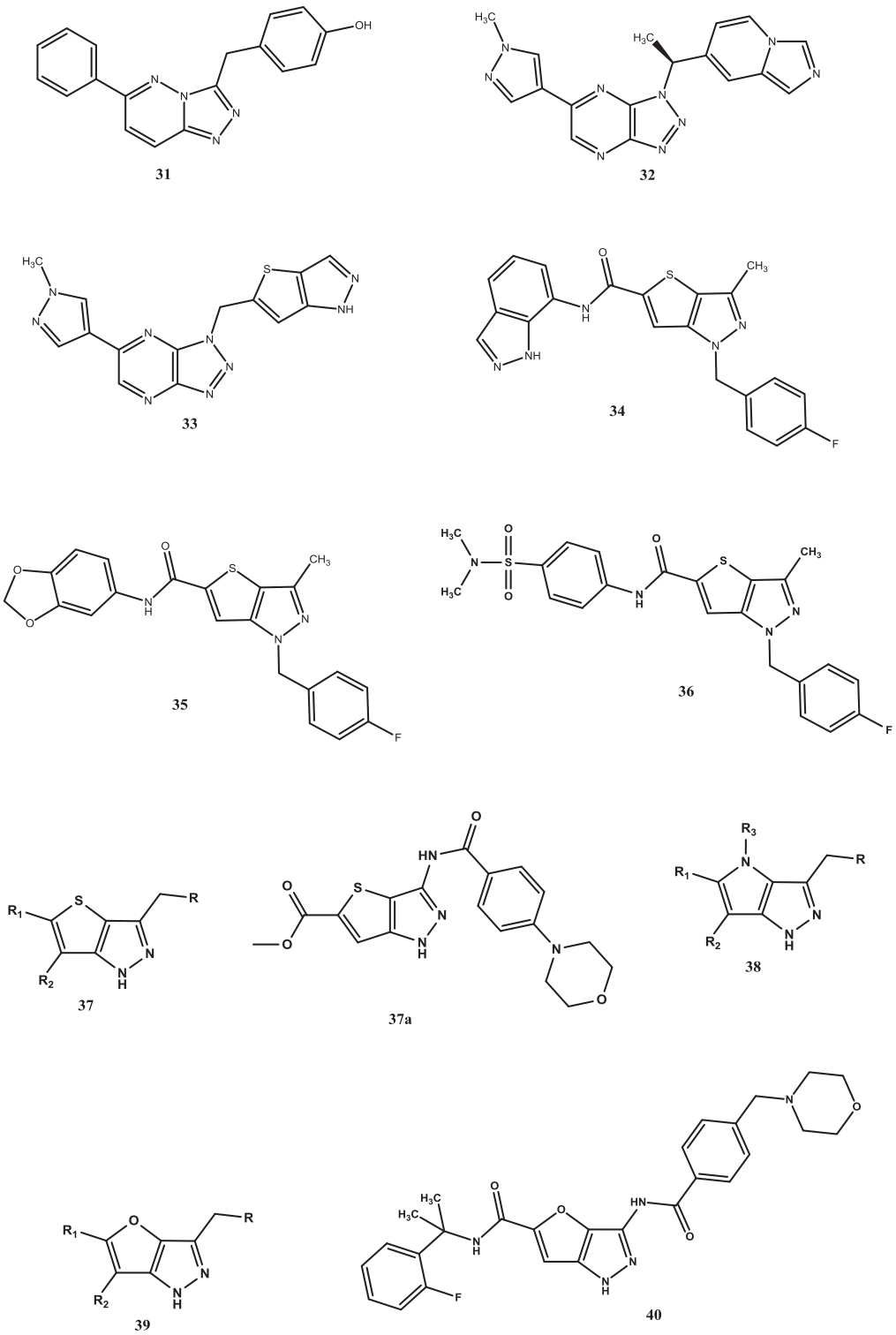
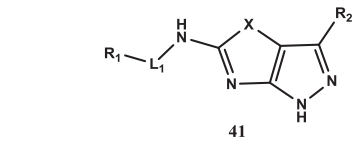



**Fig. 6.** Structures of compounds **31**–**40**.

wherein $R_1$ and $R_2$ are selected between C1–C24 alkyl, C3–C9 cycloalkyl, heterocycloalkyl, aryl, heteroaryl, arylalkyl and heteroarylalkyl, $R_4$ represents aryl, heteroaryl, C3–C9 cycloalkyl and heterocycloalkyl.

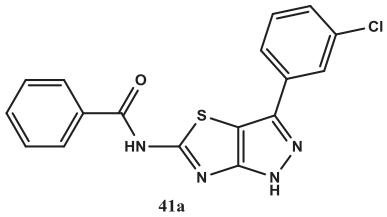
Some compounds **18** showed to inhibit Aurora-2, CDk2 and Tie2 in a sub-micromolar range such as derivative **18a** that inhibited Aurora 2, CDK2 and Tie2 with $IC_{50}$ of <50 nM, < 500 nM and <500 nM, respectively. Finally authors reported that selected compounds could be utilized to prepare medicaments useful for the treatment of cancer (Fig. 4).

Barberis et al. [16] reported the synthesis of hydrazinocarbonylthienopyrazoles **19** wherein $R_2$ = H, substituted or unsubstituted cycloalkyl, heteroaryl, etc.; $R_3$–$R_5$ are independently H, substituted or unsubstituted alkyl, alkylaryl, alkylheteroaryl; or

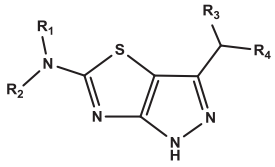


41

X = S, O, $NR_3$
$L_1$ = CO, CS, $CNR_3$, $SO_2$
$R_2$ = $R_3$ = H, OH, cyano, substituted and unsubstituted alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl.



41a



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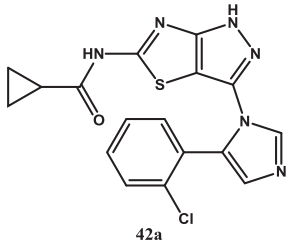
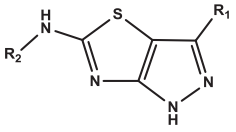


42a



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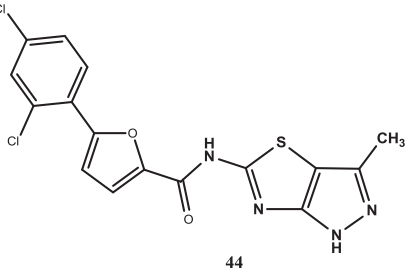

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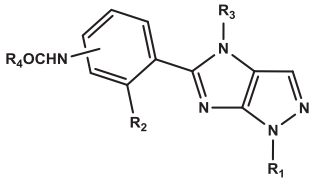
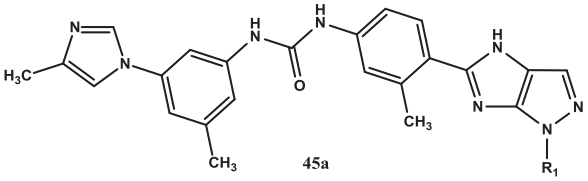
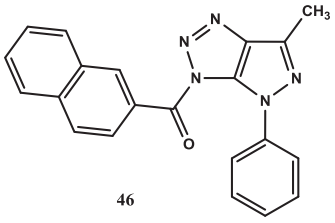


45

$R_1$ = H, Bn, PMB
$R_2$ = $R_3$ = H, Me
$R_4$ = NhAr, Ar, HetAr



45a



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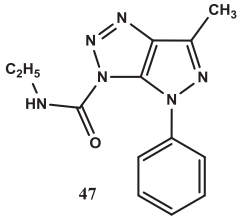


47

**Fig. 7.** Structures of compounds **41**–**47**.

$NR_3R_4$ = substituted or unsubstituted heterocyclyl; etc.

The compounds were active as protein kinase inhibitors, in particular towards Aurora 1, Aurora 2, CDK2 and Tie2 with $IC_{50}$ values in the nanomolar or micromolar range, resulting useful as anticancer agents. Compound **19a** inhibited Aurora 1, Aurora 2, CDK2 and Tie2 with $IC_{50}$ of 8 nM, 8 nM, 177 nM, and 117 nM, respectively (Fig. 4).

Doerflinger et al. [17] reported the synthesis, and the use as anticancer agent of a series of tieno[2,3-c]pyrazoles of type **20**, wherein $R_1$ represents $NHR_5$ with $R_5$ = $COR_6$ with $R_6$ = H, (un) substituted alk(en/yn)yl, (hetero)aryl, heterocycloalk(en)yl, etc.; $R_2$ is one or more substituents selected from halo, halogeno/alkyl, OH, $CO_2H$ and derivatives, $CONH_2$ and derivatives, $NO_2$, CN, etc.; $R_3$, $R_4$ are substituted or unsubstituted alkyl, $R_3CR_4$ are substituted or unsubstituted 3–6 membered cycloalkyl rings (Fig. 4).

These compounds have revealed to be inhibitors of protein kinases, in particular Aurora 1, Aurora 2 and Tie 2, showing $IC_{50}$ values in the range 1 nM–10 μM. The compounds can be utilized to prepare medicaments for the treatment of cancer.

Furthermore, Akritopoulou-Zanze et al. [18] investigated the class of thienopyrazole structures as kinase inhibitors by preparing small libraries around thienopyrazole scaffold containing overall 95 compounds with an average MW of 292, an average clogP of 2.5 and an average polar surface area of 71, corresponding to the general

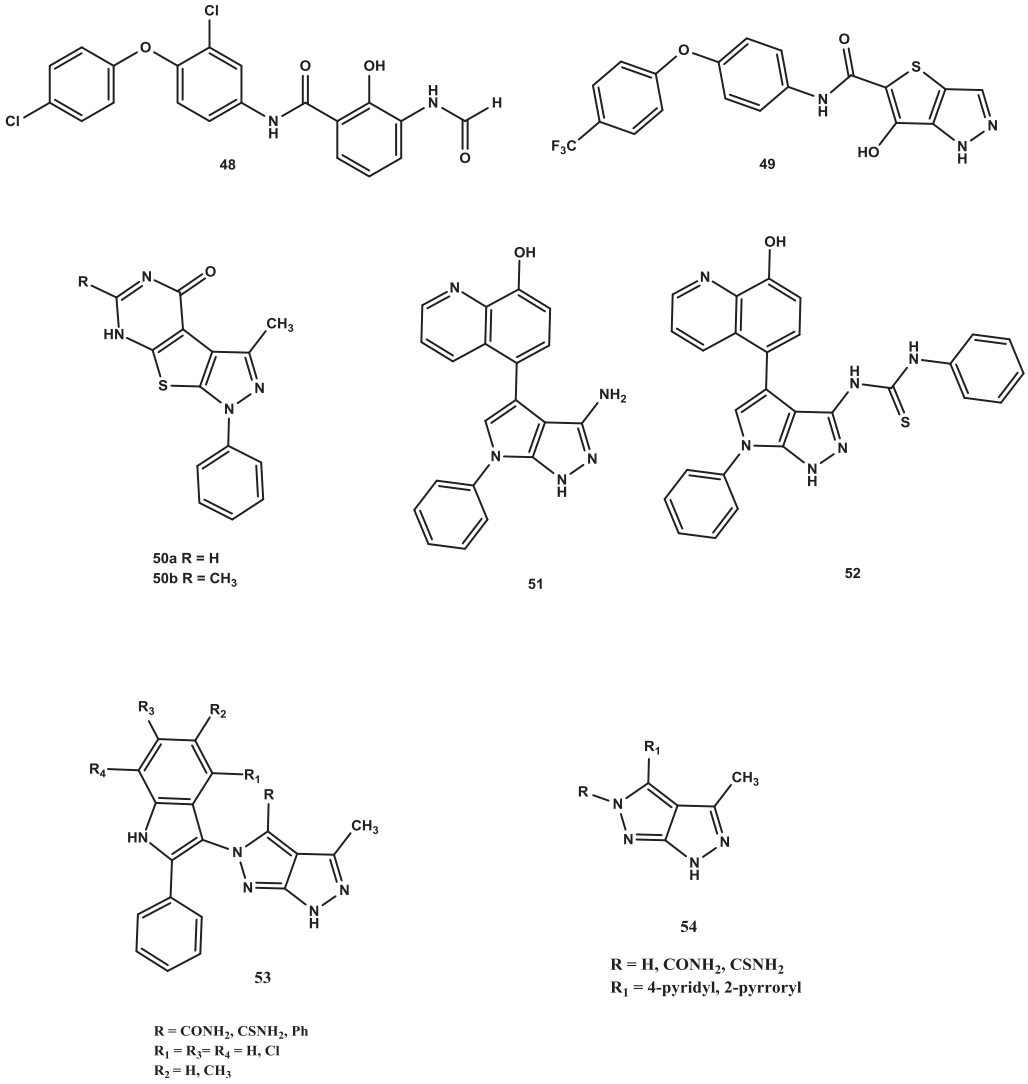



**Fig. 8.** Structures of compounds **48**–**54**.

structures of **21**, **22** and **23** (Fig. 4).

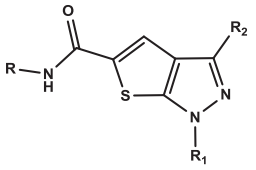
All compounds were evaluated on a panel of five kinases (KDR, P1K1, Pak4, CK2 and Akt1). For compounds **23** the evaluation was extended to a panel of 9 kinases (KDR, P1K1, Pak4, CK2 and Akt1, CDK2, p38, MK2 and COT). Numerous leads with low micromolar potency against a variety of kinase target were identified. In particular, 3-phenyl substituted thienopyrazoles exhibit the most interesting activity on KDR (Kinasi domain receptor) in a sub-micromolar concentration. The most active compound of the series was **22a**, showing an $IC_{50}$ on KDR of 0.35 μM.

Other thieno[2,3-c]pyrazoles of type **24** exhibiting anticancer activity via modulation of the activity of kinases such as FAK, KDR, SrC, Tie 2, CDK, GSK-3β, TPK1 and Aurora-2 were reported [19]. Among the synthesized compounds, 3-phenyl-1*H*-thieno[2,3-c] pyrazole-5-carboxylic acid N-benzyl-N-methylamide **24a** exhibit an activity on the various kinases, and particularly on KDR and Aurora-2, with a $K_i$ values in the range 100–5000 nM (Fig. 5).

Starting from the 1-benzyl-3-(5-hydroxymethyl-2-furyl)indazole **25** which exhibits an excellent anticancer activity due to its multiple actions including apoptosis inductions, antiangiogenesis, antiinflammation and inhibition of matrix metallo proteinases, several thieno[3,2-c]pyrazoles **1**, furo[3,2-c]pyrazoles **3** and selenolo[3,2-c]pyrazole **12**, isosteric analogs of **25**, were synthesized and tested as anticancer agents [20–22]. In particular, a series of furo[3,2-c]pyrazoles **3** were studied in deep by Chou [20]. Among the synthesized derivatives, the 1-benzyl-3-(5-hydroxymethyl-2-furyl)-5-methylfuro[3,2-c]pyrazole **26** resulted more potent than **25** with an ED50 of 5.12 μM against HL-60 cell line compared to the value of 25.27 μM of the reference compound **25**. Biological studies showed that **26** acts with an antileukemia mechanism of action different from that of **25.** It induces terminal differentiation of HL-60 cells by regulation of BCl-2 and c-Myc proteins also showing apoptosis-inducing effect. A further study carried out by the same group of research [21] to evaluate the biological mechanisms of the analogs of **25** highlighted a significant suppression of the expression of HER2 and a consequent inhibition of cell proliferation as well as apoptosis induction in HER2-overexpressing cancer cells such as MDA-MB-435/HRE2 and MCF7/HER2 (Fig. 5).

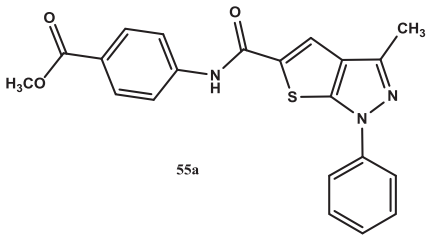
Among the analogs, compound **26**, resulted the more active making it the most promising compound for further study. As part of their research for potential anticancer drug candidates among **25** analogs, the anticancer activity and the biological mechanism of new selenolo[3,2-c]pyrazoles **12** were investigated [22].

In particular, the 3-substituted selenolo[3,2-c]pyrazoles **27** and

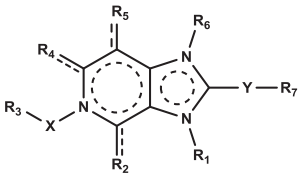


55

R = substituted and unsubstituted aryl and heteroaryl
$R_1$ = substituted and unsubstituted aryl and heteroaryl
$R_2$ = alkyl



55a



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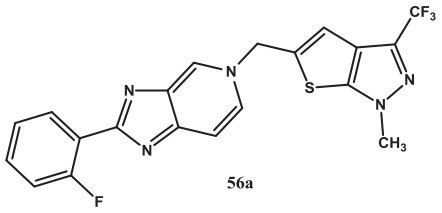
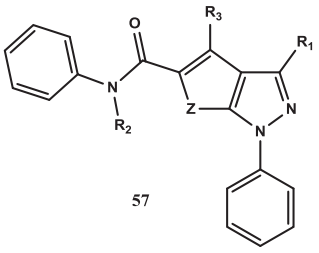


56a



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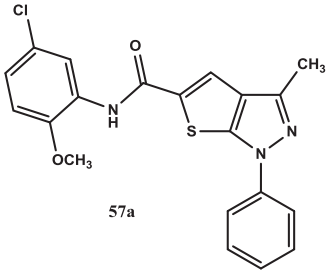

57a

**Fig. 9.** Structures of compounds **55**–**57**.

**28** were synthesized and evaluated for their cytotoxicity against NCI–H266 non-small cell lung cancer and A-498 renal cancer cell lines. The most active compounds resulted **28a** which, unlike the furo[3,2-c]pyrazole **26**, it seems to have a biological mechanism similar to that of **25** (Fig. 5).

Yamashita et al. [23] reported the synthesis of active new pyridine derivatives in the treatment of cancer and arthritis acting as inhibitors of the three isoforms of protein kinase B (Akt). Among the synthesized compounds, some derivatives, such as compound **29**, showed the thienopyrazole moiety in the structure. The use of these derivatives in the manufacture of an antitumor medicament has been described.

Similarly to compounds **29**, Su et al. [24] synthesized and evaluated for their inhibitory activity of c-Met kinase pathway (hepatocyte growth factor receptor) new triazolopyridines and triazolopyrazines substituted with the 1*H*-thieno[3,2-c]pyrazolo moiety. Among the 5-((1*H*-[1,2,3]triazolo[4,5-b]pyrazin-1-yl) methyl)-1*H*-thieno[3,2-c]pyrazoles **30**, the most active compounds of the series were **30a**–**d** with an $IC_{50}$ against the enzyme within the range 0.001–0.1 μM (Fig. 5).

Another example of thieno[3,2-c]pyrazoles **1** was shown by Jia et al. [25] that studied the structure-activity relationship of some triazolopyrazines. Docking studies on compound **31**, the first c-Met inhibitor of the series, revealed a unique binding mode: a bent "u-shaped" conformation bounded to the activation loop of c-Met kinase. Compound **31** forms a hydrogen bond between the backbone NH of Met1160 and the oxygen of the phenol, the hinge binder, a hydrogen-bonding interaction between N1 of the inhibitor and the backbone NH of Asp1222, and a π–π stacking interaction between the triazolopyridazine core and Tyr1230 (Fig. 6).

To investigate the structure-activity relationship of this molecules, the structure **31** were divided in two parts: a core represented by the [1,2,4]triazolo[4,3-b]pyridazine nucleus and the hinge piece represented by the 4-methylphenol. Modification on the core structure to have an N possible for binding with Asp1222 of the c-Met enzyme as well as the substitution of the hinge piece with a quinoline moiety to retain the hydrogen bond with the backbone NH of Met1160, led to compound **32** with high biochemical potency toward c-Met ($IC_{50}$ of 0.005 μM) (Fig. 6).

To introduce further structural differentiation from c-Met inhibitors, new chemical moieties were introduced to substitute the quinolone hinge motif. Several heterocycles were investigated and the thieno[3,2-c]pyrazole **33** greatly improved the potency more than 30-fold with an $IC_{50}$ of 0.006 μM in enzyme and 0.019 μM in cell.

This proved the position of N on the hinge piece is crucial for the binding with the backbone NH of Met1160, and the NH of **33** may also contribute to the binding as a potential hydrogen donor (Fig. 6).

Novel thieno[3,2-c]pyrazoles of type **1** were also identified by Williams [26] as modulator of Rac-GTPase mediated disorder and for treatment of chronic myelogenous leukemia. The discovery of such compounds is based on a screening method which test the inhibitory activity on cancer cells and alternatively or in addition the inhibition of the activation of the Rac-GTPase as well as the docking of a large number of structures onto the Rac-GTPase protein. Five lead compounds were identified by this screening and, among these, the 1-(4-fluorobenzyl)-N-(1*H*-indazol-7-yl)-3-methyl-1*H*-thieno[3,2-c]pyrazole-5-carboxamide **34**. A western

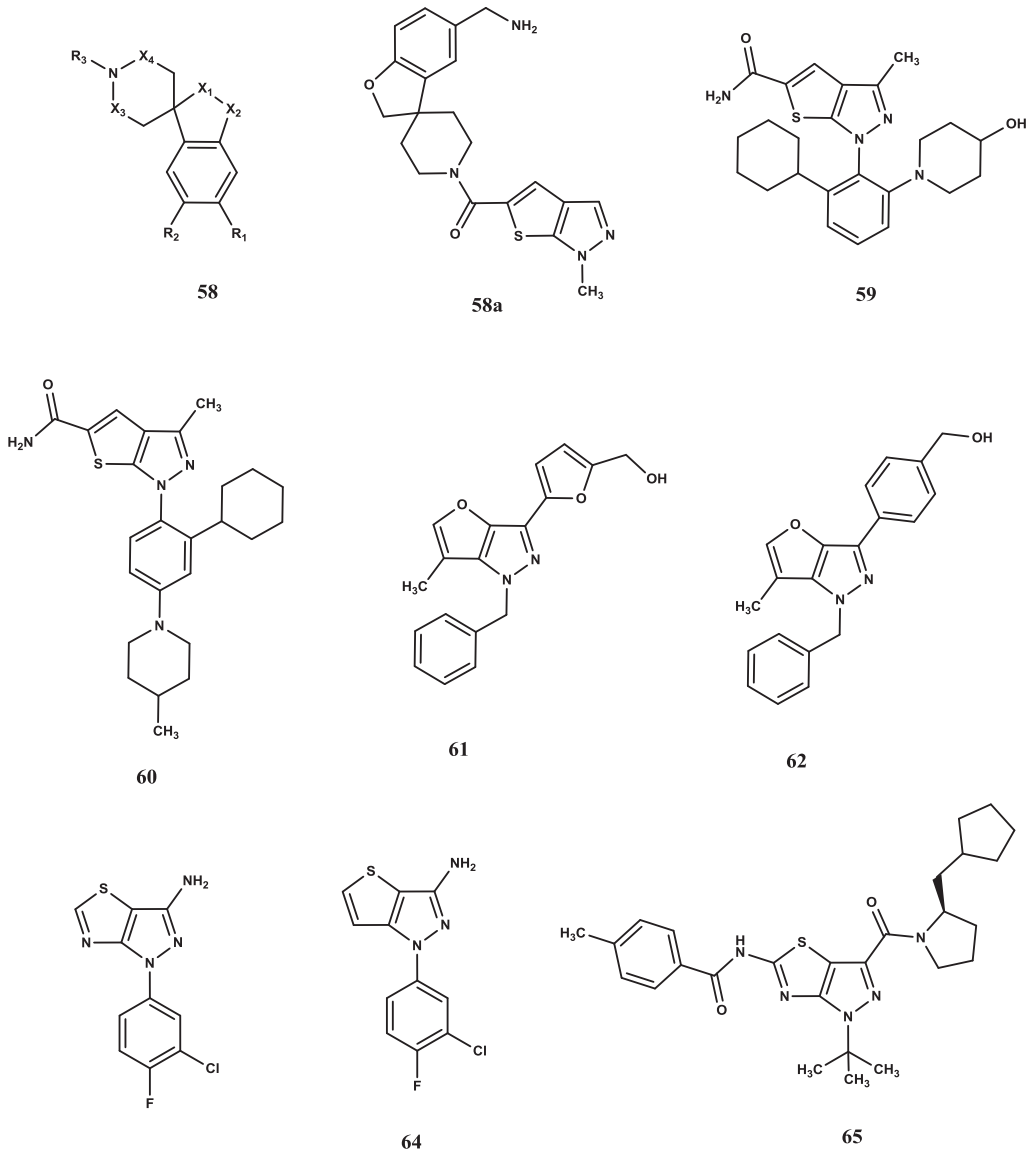


**Fig. 10.** Structures of compounds **58**–**65**.

blot analysis showed how the presence of **34** results in a reduction of Rac-1-GTPase protein. Furthermore, REH, SEM, MV411, Jurkat, Raji, Nomo-1 and Naim6 leukemia cell lines resulted very sensitive at 20 μM concentration toward **34**. Starting from this lead, the two new thieno[3,2-c]pyrazole analogs **35** and **36** were identified resulting good inhibitors of MV411, Jurkat, Raji, Nomo-1, Naim6 and SEM, MV411, Jurkat, Raji leukemia cell lines respectively at 20 μM (Fig. 6).

1*H*-thieno[3,2-c]pyrazoles **37**, 4-substituted-1,4-dihydropyrrolo [3,2-c]pyrazoles **38**, and 1*H*-furo[3,2-c]pyrazoles **39**, are further heterobicyclic pyrazoles described by Tonani et al. as protein kinase (PK) inhibitors (Fig. 6) [27,28].

All the synthesized compounds were tested for evaluating their inhibitory activity against different PKs. The best activities were described for 1*H*-thieno[3,2-c]pyrazoles **37**. In particular, methyl 3-[4-(morpholin-4-yl)benzoylamino]-1*H*-thieno[3,2-c]pyrazole-5-carboxylate **37a** inhibited Aurora-1 kinase with $IC_{50}$ less than 100 nM, 1000 nM, and 500 nM against Aurora-2, cdc7, and PAK-4 respectively.

Finally, an interesting PKs inhibitory activity, in particular against Aurora kinase 2, has also been found for some 1*H*-furo[3,2-c]pyrazoles [29,30]. The N-(2-(2-fluorophenyl)propan-2-yl)-3-(4-(morpholinomethyl)benzamido)-1*H*-furo[3,2-c]pyrazole-5-carboxamide **40**, the most active compound of the series, showed an $IC_{50}$ of 1 nM against Aurora-2 and an antiproliferative activity with $IC_{50}$ of 2 nM on HCT-116 cell line (Fig. 6).

### *2.2. Pyrazoles condensed with a five membered heterocycle having two heteroatoms*

Pyrazoles fused with another five membered heterocycle having two heteroatoms were described by Bounaud et al. [31]. They reported the synthesis and use as protein kinase modulators of 1*H*-pyrazolo[3,4-d]thiazole, 1*H*-pyrazolo[3,4-d]oxazole and 4-substituted-1,4-dihydroimidazo[4,5-c]pyrazole having the formula **41**. Compounds **41** were tested to determine their ability to modulate protein kinase, bind protein kinase as well as for prevent cell growth or proliferation.

Some pyrazolo-thiazole ($L_1 = CO$) compounds showed to inhibit FLT3, Abl, Abl315I, AblY393F, MET, RON activity with $IC_{50}$ values in

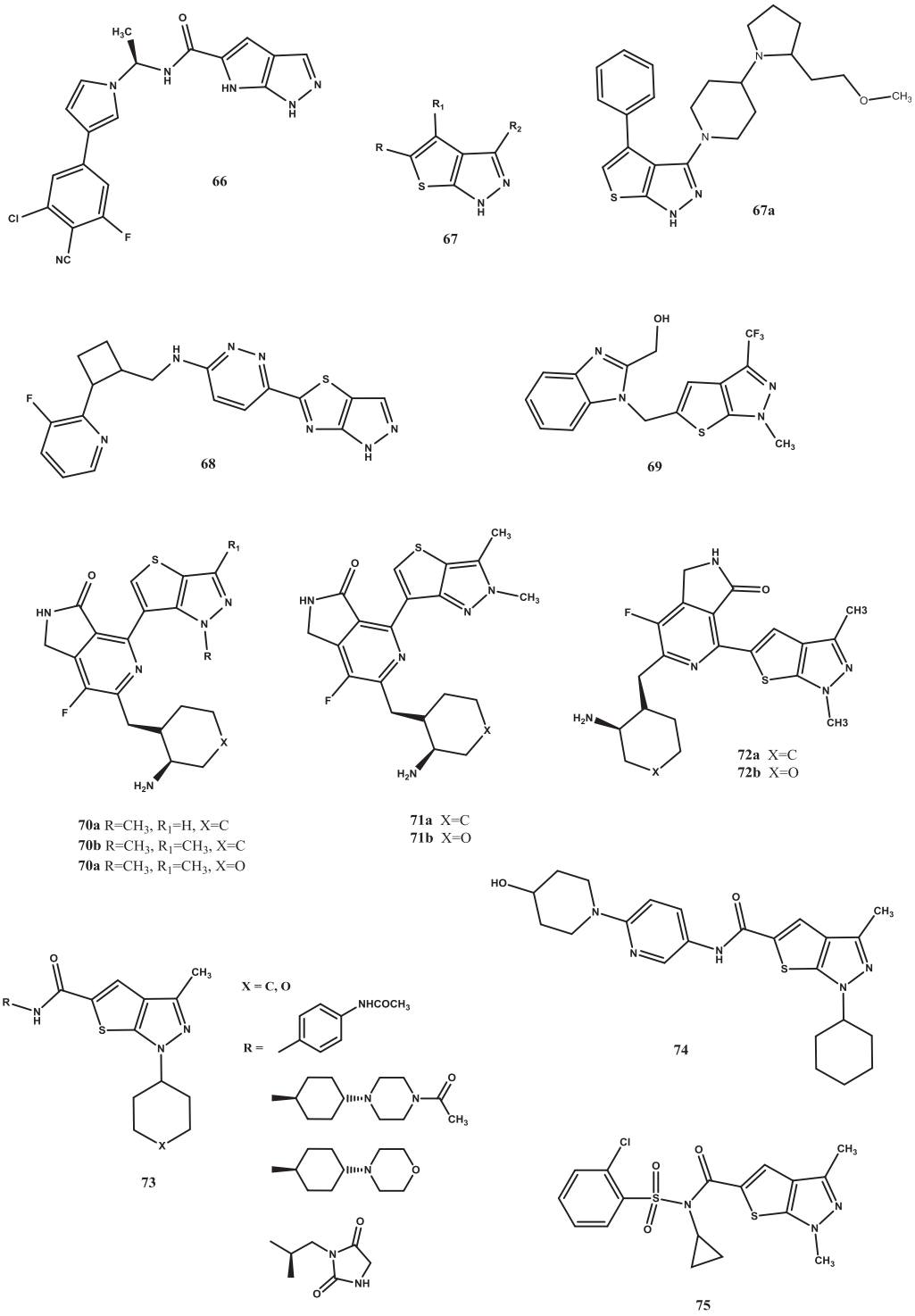


**Fig. 11.** Structures of compounds **66**–**75**.

the micromolar and nanomolar range. Moreover, the growth inhibitory activity in the micromolar range against MV4 and THP cell lines was also showed. As example, N-[3-(3-chlorophenyl)-1*H*-pyrazolo[3,4-d]thiazol-5-yl]benzamide **41a**, has an $IC_{50}$ less than 1 μM for FLT-3 kinase (Fig. 7).

The above authors reported also that pharmaceutical compositions containing bicyclic pyrazolo-fused kinase modulators may be used for treatment of diseases mediated by kinase activity, in particular different types of tumors and other diseases based on abnormal cell growth.

Other examples of 1*H*-pyrazolo[3,4-d]thiazoles were the compounds **42** synthesized by Bounaud et al. [32] wherein $R_1$ and $R_3$ are independently H, substituted or unsubstituted alkyl, aryl and heteroaryl, $R_2$ and $R_4$ represent independently substituted or unsubstituted alkyl, cycloalkyl, heterocycloalkyl, aryl and heteroaryl. A potent kinase modulator activity was described for these

derivatives, for which $IC_{50}$ or $K_i$ values less than 1 nM are reported.

Among the derivatives described in the invention, the cyclopropanecarboxylic acid {3-[5-(2-chlorophenyl)-imidazol-1-yl]-1*H*-pyrazolo[3,4-d]thiazol-5-yl}-amide **42a** showed the better activity against T315I, Aurora A, Met and CDK4/CycD1 (Fig. 7).

To the group of 1*H*-pyrazolo[3,4-d]thiazoles, also belong compounds **43** [33] in which $R_1$ and $R_2$, independently are substituted or unsubstituted alkyl, aryl, heteroaryl, cycloalkyl and heterocycloalkyl (Fig. 7).

Some compounds showed a constant inhibition against CDK4 and CDK2 of about 5 μM or less. The patent also proposes the use of this compound for therapeutic purposes or for preventive side effects associated with antiproliferative therapeutic agents.

Other small molecule containing the 1*H*-pyrazolo[3,4-d]thiazole scaffold are inhibitors or modulators of HePTP that can be used for pre-leukemia condition, leukemia, as well as acute myelogenous leukemia and myelodysplastic syndrome [34]. 5-(2,4-Dichlorophenyl)-N-(3-methyl-1*H*-pyrazolo[3,4-d]thiazol-5-yl) furan-2-carboxamide **44** is an example of such compounds. It shows $IC_{50}$ value for HePTP of 2.12 μM and an inhibition against HePTP of 92.4% at 20 μM (Fig. 7).

Other Pyrazoles condensed with a five membered heterocycle having two heteroatoms are the 1,4-dihydropyrazolo[4,5-c]imidazolephenyl derivatives having the formula **45**, reported by Hana Yu et al. [35] as type II kinase inhibitors.

These compounds were tested against WM3629 human melanoma cell lines ($IC_{50}$ = 0.56–0.86 μM) and some of them showed better antiproliferative activities than the reference drug sorafenib. Moreover, some compounds showed C-Raf inhibitory activity with $IC_{50}$ values in the nanomolar range. Compound **45a** was the most active inhibitor. It was tested against a panel of 30 different kinases at a single dose concentration (10 μM) and was confirmed as a potent and selective C-Raf kinase inhibitor (Fig. 7).

### 2.3. Pyrazoles condensed with a five membered heterocycle having three heteroatoms

Pyrazolo[3,4-d][1,2,3]triazoles capable to cleave DNA after light activation were described by Manfredini et al. [36]. Two representative pyrazolo[3,4-d][1,2,3]triazoles (compounds **46** and **47**) were synthesized with the goal to obtain less cytoxic compounds able to be activated in neoplastic tissues, leading to potential less toxic antitumor agents (Fig. 7).

Compound **46** showed to inhibit PCR-mediated amplification of the human c-myc oncogene promoter after light irradiation, with an $IC_{50}$ value of 150 μM. It was also assayed against human T-lymphoid Jurkat cell line showing an $IC_{50}$ value of about 100 μM.

## 3. Antimicrobial activity

The antibacterial and antifungal activities of pyrazoles fused with a five membered heterocycle are less represented respect to the antitumor activity. The structures can be clustered into six categories (Fig. 2): thieno[3,2-c]pyrazole **1**, thieno[2,3-c]pyrazole **2**, imidazo[4,5-c]pyrazole **6,** pyrazolo[3,4-d][1,2,3]triazole **7,** pyrrolo [2,3-c]pyrazole **8** and pyrazolo[3,4-c]pyrazole **9**.

### 3.1. Pyrazoles condensed with a five membered heterocycle having one heteroatom

Starting from the structure of the N-(3-chloro-4-(4-chlorophenoxy)phenyl)-3-formamido-2-hydroxybenzamide **48**, a functional antimycin equivalent acting as potent inhibitor of the Qi site of complex III ($bc_1$), Bolgunas et al. [37] synthesized and tested some azole-fused salycilamides and the thieno bioisostere **49** (Fig. 8), designed to mimic compound **48**. Among the synthesized compounds, the 6-hydroxy-*N*-{4-[4-(trifluoromethyl)phenoxy] phenyl}-1*H*-thieno[2,3-*c*]pyrazole-5-carboxamide **38** (Fig. 6) resulted active as growth inhibitor against *Septoria nodorum* with a $GR_{50}$ of 11514 nM (antimycin $GR_{50}$ = 33 nM) (Fig. 8).

The effects on mitochondrial electron transport were measured by two different assays: the FMET2-3 assay to measure the inhibition versus both complex 2 (succinate dehydrogenase) and complex 3 ($bc_1$ complex) and the FMET2 assay to measure the inhibitory activity against complex 2 only. Compound **50** resulted inactive against complex 2 in the FMET2 assay ($IC_{50}$ higher than 30,000), so the activity shown in FMET2-3 assay ($IC_{50}$ = 26 nM) is due only to activity at the $bc_1$ complex. Despite its intrinsic potency, the *in vivo* activity against *S. nodorum* is much lower than expected, possibly due to poor cell penetration capability. Another example of antimicrobial thienopyrazoles were reported by Rabie et al. [38] which described the synthesis of pyrazolo[4′,3':4,5]thieno[2,3-d] pyrimidin-4-ones **50a**,**b** (Fig. 8).

Shawkat [39] reported the synthesis and the antimicrobial activity of some 2-amino-4-(8-quinol-5-yl)-1-(p-tolyl)-pyrrole-3-carbonitriles. Among the synthesized compounds, the two pyrrolo[2,3-*c*]pyrazoles **51** and **52** are described (Fig. 8).

Compounds **51** and **52** show *in vitro* antibacterial activity against *Bacillus cereus*, and *Escherichia coli* with a diameter of inhibition zone of 19–41 mm as well as *in vitro* antifungal activity against *Aspergillus Flavus* and *Stachybotrys atra* with a diameter of inhibition zone of 7–34 mm. Their activity resulted similar or slightly higher to tioconazole taken as standard drug.

### 3.2. Pyrazoles condensed with a five membered heterocycle having two heteroatoms

Among the pyrazoles fused with another five membered heterocycle having two heteroatoms, the pyrazolo[3,4-c]pyrazole system **9** is the most represented with three references by Mallikarjuna Rao et al. [40–42]. In particular, they investigated [40,41] the antimicrobial activity of pyrazolo-pyrazole derivatives **53** bearing the indole moiety for their antibacterial activity against *E. coli*, *Bacillus subtilis* and *Streptococcus pneumonia* as well as the antifungal activity against *Aspergillus niger* and *Candida albicans*. The best antibacterial as well as antifungal activities were obtained when R was $CONH_2$ or $CSNH_2$ and the indole moiety was substituted with chlorine atoms (Fig. 8).

They [42] also described the antimicrobial activity of pyrazolo-pyrazole derivatives **54** bearing the pyridine and pyrrolo moiety, for their antibacterial activity against *E. coli*, and *S. pneumonia* as well as the antifungal activity against *A. niger* and *C. albicans*. Compounds **54** exhibits very good activity against antibacterial and antifungal activity expressed as zone of inhibition ranging from 8 to 14.2 mm for *E. coli*, 14–2 mm for *S. pneumonia*, 10–16 mm for *A. niger* and 10–17 mm for *C. albicans*. Their activity resulted similar or slightly higher to Gentamicin (antibacterial activity) and slightly lower to fluconazole (antifungal activity) taken as standard drugs (Fig. 8).

## 4. Antiviral activity

Pyrazoles condensed with a five membered heterocycle endowed with antiviral activity can be clustered into three categories (Fig. 2): thieno[2,3-c]pyrazoles **2**, furo[2,3-c]pyrazoles **10** and pyrrolo[3,4-c]pyrazoles **11**.

### 4.1. Pyrazoles condensed with a five membered heterocycle having one heteroatom

To this class of pyrazoles fused with a five membered

heterocycle having one heteroatom, belong the: thieno[2,3-c]pyrazoles **2** and furo[2,3-c]pyrazoles **10**, even if the first are most numerous [43–45]. In particular, Chunduru et al. [43] reported compounds of type **55** useful for the treatment and prophylaxis of viral infections, particularly those viral infections and associated diseases caused by Hepatitis C virus (HCV). The ability to inhibit the functions of the non structural protein 4B (NS4B) of this virus, essential to viral replication, is the basis of the mechanism of action related to compounds **55** (Fig. 9).

Among the large number of derivatives described, the methyl 4-(3-methyl-1-phenyl-1*H*-thieno[2,3-c]pyrazole-5-carboxamido) benzoate **55a**, interacts with the NS4B protein with a binding constant (Kd) of 1 μM, it produces a dose-dependent inhibition of intracellular NS5A levels with an $EC_{50}$ equal to 3.15 μM and cellular cytotoxicity ($CC_{75}$(CV) of 50 μM) [43]. The thieno[2,3-c]pyrazole moiety is still present in a series of imidazo[4,5-c]pyridine derivatives of type **56** containing at least 3 or 4 double bonds (dotted lines) and differently substituted in $R_1$–$R_7$ described as antiviral agents useful in the treatment of hepatitis C virus by Puerstinger et al. [44].

Among the large number of synthesized and tested compounds, only one have the thieno[2,3-c]pyrazole moiety in $R_3$, the 5-((2-(2-fluorophenyl)-5H-imidazo[4,5-c]pyridin-5-yl)methyl)-1-methyl-3-(trifluoromethyl)-1*H*-thieno[2,3-c]pyrazole **56a**. All compounds of type **56**, evaluated in an anti-HCV/Replicon assay system, showed an antiviral activity of at least 1 μM (Fig. 9).

The thieno[2,3-c]pyrazole system was also present among a large number of compounds reported by Silverman [45], having general formula **57** wherein $R_1$ and $R_3$ are independently hydrogen, C1–C5 alkyl or a C1–C5 haloalkyl groups and $R_2$ hydrogen, C1–C5 alkyl group. They showed RNase L activity with antiviral activity against Parainfluenza virus 3, picornavirus and encephalomyocarditis virus. Among the synthesized compounds, derivative **57a** increased the RNase L activity with an $EC_{50}$ of 48 μM. In this report structures having the furo[2,3-c]pyrazole system **3** were reported but no activity were described (Fig. 9).

## 5. Antiinflammatory activity

The anti-inflammatory activity of pyrazoles fused with a five membered heterocycle can be clustered into five categories (Fig. 2) such as thieno[3,2-c]pyrazoles **1**, thieno[2,3-c]pyrazoles **2**, furo[3,2-c]pyrazoles **3**.

### *5.1. Pyrazoles condensed with a five membered heterocycle having one heteroatom*

Pyrazoles fused with a thiophene ring having antiinflammatory activity were described [46–48]. In particular, Costanzo et al. [46] reported the synthesis and the biological activity of some spiropiperidines **58**. Compounds **58** resulted triptase inhibitors useful for treating triptase mediated disorders, including inflammatory diseases associated with the respiratory tract such as asthma and allergic rhinitis as well as other immunomediated inflammatory disorders such as rheumatoid arthritis, conjunctivitis and psoriasis. Among the more than 360 compounds described, the thienopyrazole derivate **58a** showed triptase inhibitory activity with an $IC_{50}$ of 1.2 μM (Fig. 10).

Thieno[3,2-c]pyrazoles ASB16165 (1-Cyclohexyl-N-[6-(4-hydroxy-1-piperidinyl)-3-pyridinyl]-3-methyl-1*H*-thieno[2,3-c] pyrazole-5-carboxamide) **59** and SUN11817 (1-Cyclohexyl-N-[3-fluoro-4-(4-methyl-1-piperazinyl)phenyl]-3-methyl-1*H*-thieno [2,3-c]pyrazole-5-carboxamide) **60** [47,48], were studied as anti-inflammatory agent able to inhibit the phosphodiesterase 7A. In these reports, possible roles in skin inflammation such as psoriasis as well as liver injury like hepatitis are considered (Fig. 10.).

Finally, Chien [49] described the computational study on cyclooxygenase inhibitors with the aim to develop new COX2-selective inhibitors. Among the compounds selected for the virtual screening, the furo[3,2-c]pyrazoles **61** and **62** were considered. According to binding free energies, compounds **59** and **60 s**howed no significant selective effect on COX-1 and COX-2 (Fig. 10).

## 6. Activity against CNS disorders

Pyrazoles fused with the thiophene or 1,3-thiazole to form the thieno[3,2-c]pyrazole **1** or pyrazole[3,4-d]thiazole **5** systems respectively (Fig. 2), endowed with some activities against CNS disorders were described [50,51].

Conn et al. [50] reported the synthesis and the biological activity of several classes of pyrazoles fused with a five member heterocycle useful as allosteric potentiators or positive allosteric modulators of the metabotropic glutamate receptor subtype 4 (mGluR4). These compounds could be used in treating neurological and psychiatric disorders or other diseases associated with glutamate dysfunction. Among the about 130 described compounds, the N-(3-chloro-4-fluorophenyl)-1*H*-pyrazolo[3,4-*d*][1,3]thiazol-3-amine **63** and the 1-(3-chloro-4-fluorophenyl)-1*H*-thieno[3,2-*c*]pyrazol-3-amine **64** were described. Compound **63** showed an $EC_{50}$ value of 1180 nM for potentiation of mGluR4 activity (Fig. 10).

The activity of the thieno[3,2-c]pyrazole system was also investigated by Yu et al. [51]. They considered the mammalian proline transporter (PROT) which is a high affinity $Na^+/Cl^-$-dependent transporter expressed in specific regions of the brain. Previously, only peptides were found to inhibit this transporter but no non-peptide small molecule inhibitors have been described. By screening libraries of small molecules of approximately 300,000 molecules, a large number of compounds were found to have inhibitory activity and in particular, compounds LP-403812 **65** showed an $IC_{50}$ of 0.1 μM on both recombinant human and mouse PROT; it also showed high selectivity against PROT being inactive on glycine and dopamine transporters (Fig. 10).

## 7. Other activities

Although less represented, a number of other biological activities were reported for the pyrazole fused with a five membered heterocycle.

In particular, Tormakangas et al. [52] described the synthesis of a large number of non-steroidal carboxamides including pyrrolo[2,3-c]pyrazoles **11** such as compound **66**, having utility as tissue-selective androgen receptor modulators towards patients requiring androgen receptor antagonist therapy. Compound **66**, like all other carboxamides which possess androgen receptor antagonism, is therefore useful to the prevention or the treatment of several androgen receptor depending diseases such as tumors and in particular prostate cancer. Thieno[2,3-c]pyrazoles **1** with general structure **67** were reported by John et al. [53] as inhibitors of $K_{ir}3.1$ and $K_{ir}3.4$ potassium channels subunits subtypes, finding application in the treatment of cardiovascular diseases, for example against fibrillations, arhythmias and anomalies of rhythm and conduction of the contractile impulse but also against neurological disorders as pain, depression, anxiety and epilepsy and against cancer. In most cases these compounds, such as compound **67a**, showed an $IC_{50}$ in the $K_{ir}3.1/3.4$ electrophysiology method of less than 500 nM (Fig. 11).

In the context of the research of new compounds able to modulate skeletal muscle contractility, many amino-pyridazines were synthesized [54]. Among these, compound **68** containing the pyrazolo[3,4-d]thiazolo **5** moiety resulted a fast fiber activator

with an AC1.4 (the concentration at which the compound increases of 40% the enzymatic activity of muscle myofibril preparations) value less than 1 μM (Fig. 11).

Yet, a new class of TNFα modulating benzimidazoles was synthesized by Brooking et al. [55]. They may be useful in therapy in the treatment of adverse inflammatory and autoimmune, neurological and neurodegenerative, pain and nociceptive, cardiovascular, metabolic, ocular and oncological disorders. Among the more than thousands synthesized benzimidazoles, the (1-((1-methyl-3-(trifluoromethyl)-1*H*-thieno[2,3-c]pyrazol-5-yl)methyl)-1*H*-benzo[d]imidazol-2-yl)methanol **69** were described. No biological data for each individual compound has been reported. The authors claim that, when tested in the inhibition of TNFα-induced NF-kB activation assay, all the compounds exhibit $IC_{50}$ values of 50 μM or better (Fig. 11).

Moreover, Nie et al. [56] described the synthesis of new 6-aza-isoindolin-1-one derivatives bearing, in some cases, the thieno[3,2-c]pyrazole **1** and thieno[2,3-c]pyrazole **2** moieties. Compounds **70**, **71** and **72** are in fact described together hundred other derivatives as inhibitors of spleen tyrosine kinase (SYK). The authors state the usefulness of these compounds to treat disorders involving immune system and inflammation. Compounds **70a–c**, **71a**,**b** and **72a**,**b** exhibit very good SYK inhibition ($pIC_{50}$) ranging from 8.9 to 10 μM (Fig. 11).

Other examples of thieno[2,3-c]pyrazoles **2** having the structure **73**, directed to treatment of addictions and primary impulse-control disorders using phosphodiesterase 7 (PDE7) inhibitors, were reported [57,58]. In particular the compound ABS16165 **74** [57], showed potent inhibitory activity to recombinant human PDE7A, with an $IC_{50}$ value of 15 nM and inhibited CD3/CD28-triggered proliferation of the cells. Finally, fungicide and nematocide activities were described for N-cyclopropyl-sulfonylamide derivatives [59,60]. Among the about 700 molecules described, compound **75** was reported, even if it is not among the molecules with the highest activity (Fig. 11).

## 8. Conclusion

The main objective of this review is focused to the bicyclic systems, based on the pyrazole nucleus fused with a five membered heterocycle, as templates of a large number of compounds. These compounds exhibit a wide spectrum of pharmacological proprieties such as antitumor, antimicrobial, antiviral, anti-inflammatory, CNS disorders regulating, androgen receptor modulating, $K_{ir}3.1$ and $K_{ir}3.4$ potassium channels (subunits) subtypes inhibitory and skeletal muscle contractility modulating activity, and can surely serve the purpose to be considered as potential useful drugs.

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