# Indenopyrazole as a Privileged Structure in the Development of Anticancer Agents

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**Abstract:** A Privileged structureis a molecular scaffold that can provide potent and selective ligands for a range of different biological targets through modification of functional groups. Indenopyrazole is a three ringed heterocyclic structure consisting of a benzene ring, a central 5-membered ring and a pyrazole ring. As a privileged structure, indenopyrazole has been extensively used in recent years in the design of anticancer agents with versatile targets. A number of indenopyrazole derivatives displayed potent anticancer activities as checkpoint kinase 1, epidermal growth factor receptor, vascular endothelial growth factor, platelet-derived growth factor receptor, cyclin-dependent kinases, and tubulin polymerization inhibitors, among many others. This review will summarize the recent development of indenopyrazoles as anticancer agents, discuss their SARs.

**Keywords:** Checkpoint kinase 1, cyclin-dependent kinases, epidermal growth factor receptor, hypoxia-inducible factor 1, indenopyrazoles, inhibitors, platelet-derived growth factor receptor, privileged structures, tubulin polymerization, vascular endothelial growth factor.

## 1. INTRODUCTION

Molecular frameworks that can provide useful ligands for multiple types of receptors or enzyme targets throughproper structural modifications are regarded as privileged structures. In additionto the versatile binding properties, privileged structures also displayfavorable drug-like properties. These merits make the privileged structures a useful concept for the rational design of new lead drug candidates and contribute to the increasing hit rates at the targets. Over the past two decades, the privileged structures have become a fruitful strategy in drug discovery processes [1-3].

Indenopyrazole framework is a three-ringed heterocyclic structure consisting of a benzene ring, a central 5-membered ring and a pyrazole moiety. As shown in Figure **1**, the pyrazole portion may tautomerize and a indenopyrazole could exist in two tautomeric forms.

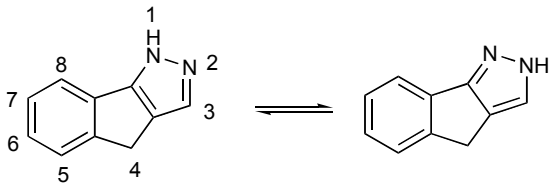


**Figure 1:** The tautomers of indenopyrazole.

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Since its first preparation in 1965 [4], indenopyrazoles have shown diverse biological activities, such as anti-psychotic [5], anti-mycobacterial [6, 7], and anticancer. In recent years, indenopyrazoles are widely used as the “privileged structure” in the design of anticancer agents targeting multiple tumor targets. In theis review, we will summarize the progresses of the privileged indenopyrazole structures as the inhibitors of checkpoint kinase 1, epidermal growth factor receptor, vascular endothelial growth factor, platelet-derived growth factor receptor, cyclin-dependent kinases, tubulin polymerization, and hypoxia-inducible factor 1,discuss their SARs.

## 2. CHECKPOINT KINASE 1INHIBITORS

Checkpoint kinase 1 (CHK-1) is a serine/threonine proteinkinase that plays a crucial role in DNA damage-induced checkpoints [8, 9]. Inhibition of CHK-1 will abrogate S and G2/M checkpoints and force tumor cells to undergo premature mitotic entry leading to cell death. Therefore, CHK-1 has emerged as an attractive chemosensitization anticancer target [10, 11].

Indenopyrazole **1** (Figure **2**) was identified as a potent CHK-1 inhibitor ($IC_{50}$= 510 nM) via a high throughput screen [12]. The X-ray co-crystal structure of CHK-1 in complex with **1** indicates that the fluoro group locates in a region of limited volume and forms extra hydrogen bonding with the polar residues of the backbone protein. Replacement of the piperidylamino side chain at the 6 position of **1** by 4-methylcyclohexylamino maintained the enzymatic potency of **1**. In addition, the *cis*/*trans* stereochemistry

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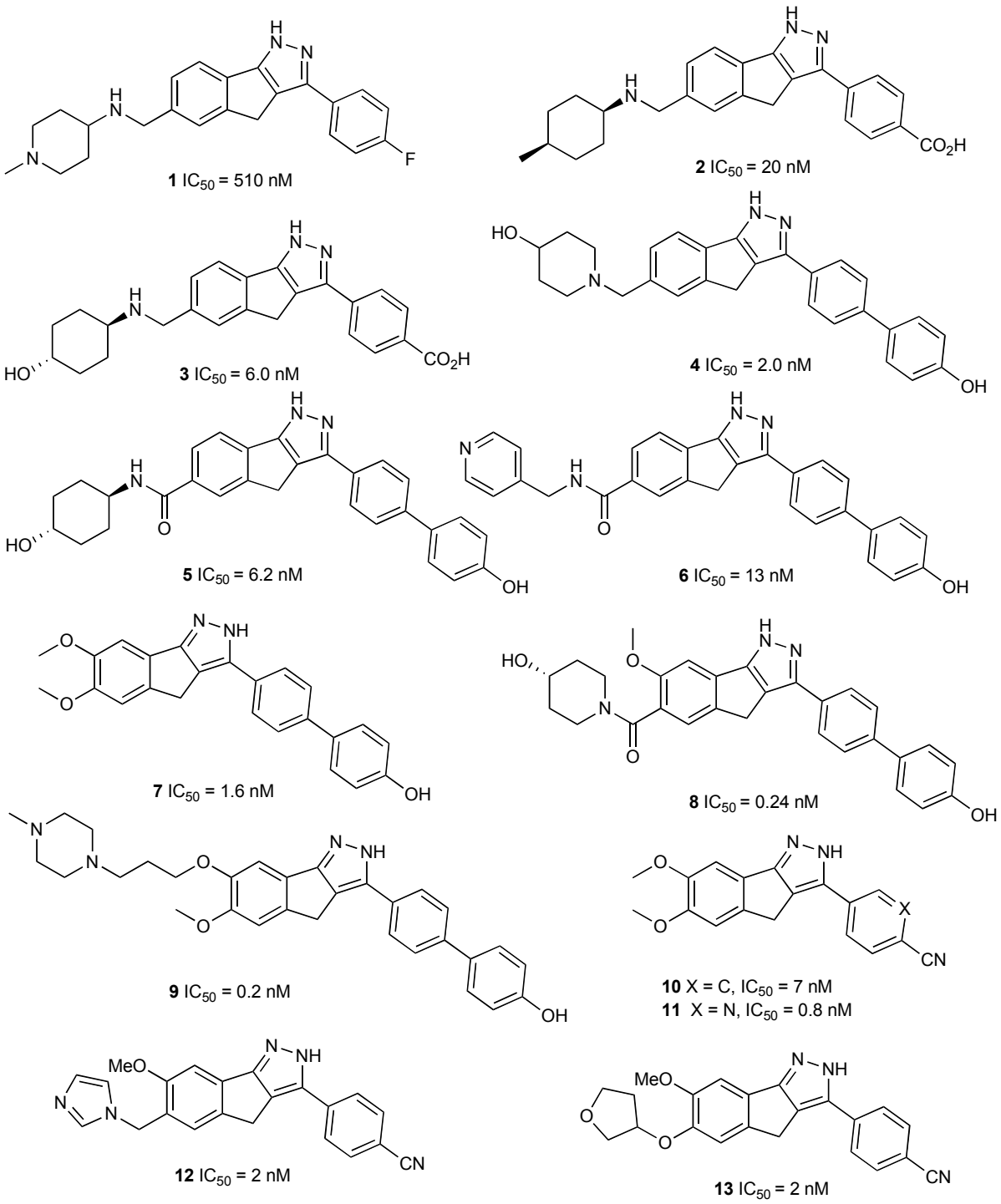



**Figure 2:** Checkpoint kinase 1 inhibitors.

of 4-methylcyclohexylamino had little effect on the potency. Further replacement of the fluoro with other functional groups, such as methyl, amino, alkylated amine, and ethoxy, decreased the inhibitory potency. However, hydroxyl group boosted the potency of **1** by fourfold. When the fluoro was replaced by a carboxylic acid, compound **2** exhibited the best results ($IC_{50}$ = 20 nM) with a 25-fold improvement from **1** [12].

In the X-ray crystallographic structure of **2** bound to the CHK-1 active site (Figure **3**), compound **2** existed in its tautomeric form. The tautomer 2NH formed two hydrogen bonding interactions with the hinge residues of CHK-1 by donating an H-bond to Glu85 and accepting an H-bond from Cys87, similar with that of compound **1**. In comparison with **1**, the carboxylic acid group bound at the polar region, accepting a key extra

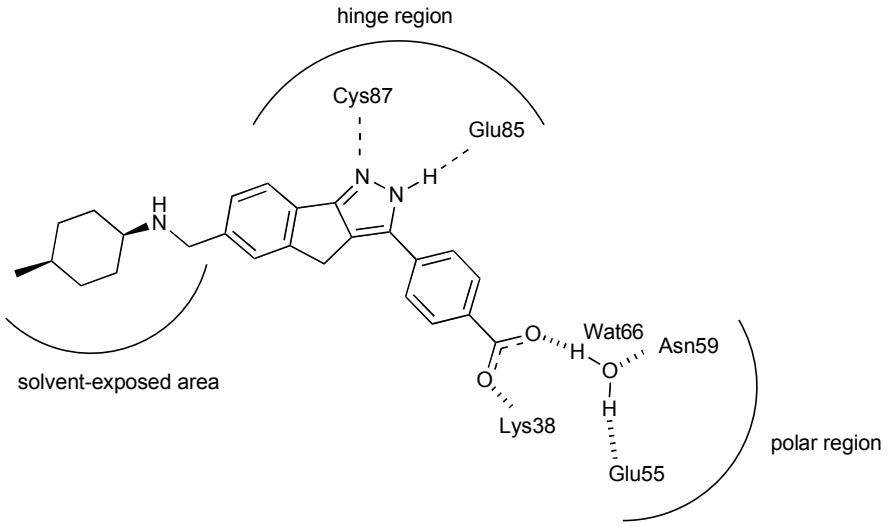



**Figure 3:** The interactions of **2** with the active site of check point kinase 1.

hydrogen bond from the positively charged amino group of Lys38, which formed a salt bridge to Glu55. Methylation of the acid of **2** would eliminate this interaction and led to a 30-fold potency loss. In addition, a bound water molecule, Wat66, was observed to make a well-defined set of three hydrogen bonds between the carboxylic acid moiety, Glu55 and Asn59. The amino side chain at the 6-position pointed to the solvent-exposed area [12].

Modification at the 6-position of the indenopyrazole ring was generally tolerated. Replacement of the 4-methyl group in 4-methylcyclohexylamino group of **2** with a *trans*-hydroxyl led to increased inhibitory potency (**3**, $IC_{50}$ = 6.0 nM). Further installing a phenol unit at the carboxylic acid position gave rise to a series of biaryl phenolic indenopyrazoles that in general had higher binding affinity than the carboxylic acid inhibitors. The most potent compound **4** had an $IC_{50}$ of 2.0 nM. Moreover, both compounds **5** and **6** were selective against other serine/threonine kinases and displayed weak $EC_{50}$s (>25 μM) when tested alone against human colon carcinoma SW620 cells, but sensitized the anti-proliferative activity of camptothecin with potentiation ratio of 4.9 for **5** and 2.0 for **6** at the concentration of 3 μM, providing extra support that CHK-1 inhibitors may have potential therapeutic application in sensitizing DNA-damaging agents to combat cancer [12].

Keeping the biaryl phenol substituent at the indenopyrazole 3-position intact, SAR studies were made by introducing different substituents at the 5-, 7- and 8-positions [13−15]. The 5-position could only accommodate smaller groups like the acetylamino ($IC_{50}$ = 5.5 nM) and $CH_2OH$ ($IC_{50}$ = 7.8 nM), while even minor substitution with a hydroxyl group at the 8-position led to significant potency loss [10]. Substituent at the 7-position was outside of the binding cavity and extended into the solvent similar to those at the 6-position. Therefore, groups at this position with variable size and length all resulted in potent inhibitors with $IC_{50}$ values ranging from 2 to 34 nM. The substitutions at both the 6- and 7-positions had some additive effect. The 6,7-dimethoxy derivative**7** was a potent CHK-1 inhibitor with an $IC_{50}$ of 1.6 nM [13]. A methoxy group at the 7-position coupled with a wide variety of solubilizing substituents containing polar N, O or OH groups at the 6-position connected via a methylene, a carbonyl or an ether bond, including the (4-hydroxy-piperidin-1-yl)-carbonyl, (2-yyrrolidin-1-yl-ethylamino)-carbonyl, (*trans*-4-hydroxy-cyclohexylamino)-carbonyl (**8**), morpholin-4-yl-carbonyl, 2-hydroxy-ethylamino-methyl, (2-pyrrolidin-1-yl-ethylamino)-methyl, (*trans*-4-hydroxy-cyclohexylamino)-methyl and 2-dimethylaminoethoxy, led to very potent CHK-1 inhibitors ($IC_{50}$< 1 nM) [13, 14]. Compound **8** exhibited an $IC_{50}$ of 0.24 nM and potentiated the anti-proliferative effect of doxorubicin in HeLa cells by at least 47-fold. A methoxy group at the 6-position together with a solubilizing group at the 7-position had similar effects, inhibiting CHK-1 with $IC_{50}$ values of subnanomolar to single digital nanomolar, represented by compound **9**. However, PK studies in mice revealed that this diaryl phenol class of CHK-1 inhibitors had high clearance, moderate bioavailability when dosed intraperitoneally, and had poor oral bioavailability [14].

Considering the hydroxyl group is potentially labile to phase II biotransformations such as *O*-

glucuronidation and *O*-sulfation, further structural modifications were made at the 3-position in order to replace the biphenyl moiety of **7**. Introducing a *p*-cyanophenyl substituent at the 3-position of **7** maintained the inhibitory potency (**10**). When the cyano group in **10** was replaced by substituted phenyl, pyridyl, morpholinyl, 4-hydroxyl piperidinyl, or a variety of five-member ring heterocyclic substituent, the CHK-1 inhibitory potency was much reduced or completely lost [15]. Replacement of the biphenyl moiety of **7** with 6-cyano-3-pyridyl group led to a potent inhibitor (**11**). Moving the pyridyl N atom of **11** to the other positions in the ring considerably decreased the activity. The introduction of an additional N atom into the pyridyl ring was also detrimental to potency. Replacement of the 6-methoxy in **10** with polar groups generated a series of analogues with low single digital nanomolar $IC_{50}$ values against CHK-1. Analogues with heteroaromatic groups at the 6-position potently inhibit CHK-1 and their potencies were moderately affected by the type of pendant ring as well as the linker length between the tricyclic core and the pendant aromatic ring. The most potent compounds **12** and **13** also showed improved potentiation ratio in the cellular assay (42-fold for **12**, 57-fold for **13**) than **10** (26-fold) [15]. Therefore, the solubilizing substituentsat the 6-position not only significantly affect the enzymatic activity but also the potentiation ratio in the cellular assay.

## 3. EGFR AND VEGFR-2 (KDR) TYROSINE KINASE INHIBITORS

The epidermal growth factor receptor (EGFR, also known as erb-B1 or HER-1) tyrosine kinase plays an important role in regulating the basic cell functions as proliferation, chemotactic migration, invasion, and avoidance of apoptosis [16, 17]. Elevated expression and constitutive activation of EGFR are associated with poor prognosis in many types of cancer. Therefore, the design of highly specific and potent EGFR inhibitors is an attractive approach to control tumor growth and metastasis [18−20]. The EGFR tyrosine kinase inhibitors, Iressa and Tarceva, have been approved for the treatment of non-small-cell lung cancer.

Vascular endothelial growth factors (VEGFs) bind in three diversified but structurally relative VEGF receptors: VEGFR-1, VEGFR-2 (human KDR, kinase insert domain-containing receptor tyrosine kinase) and VEGFR-3. VEGFR-2, a 210−230 kDa glycoprotein, is specifically expressed in vascular endothelial cells and the major regulator of VEGF-driven responses in endothelial cells, including proliferation, migration, tube formation and vascular permeability [21, 22]. Its essential role in tumor angiogenesis and progression has made the inhibition of VEGFR-2 signaling a highly attractive target for the developments of anticancer drugs [23].

Among a series of indenopyrazoles that were designed and synthesized as EGFR tyrosine kinase inhibitors by in silico high-throughput screening, compounds **14**, **16**, and **17** (Figure **4**) showed moderate inhibitory activity toward EGFR tyrosinekinase with an inhibition rate (at 1 μM) of 61%, 65%, and 50%, respectively [24]. When the amino (R) group in **14** was replaced by a hydroxyl, the resulting compound **15** did not show any inhibitory effect on EGFR. Moreover, compounds **14**, **15**, and **16** showed significant VEGFR-2 tyrosine kinase inhibition (70−88%) at 1 μM concentration. In EGFR overexpressing human epidermoid carcinoma A431 cell, indenopyrazoles **14** and **15** exhibited very potent growth inhibition, and their $GI_{50}$ values (62 and 57 nM, respectively) were much lower than that of tarceva (470 nM). The western blotting analysis suggested that the significant cell growth inhibitory activity of **14** and **15** toward A431 cells might be mainly caused by regulation of CDK signaling pathway rather than the EGFR tyrosine kinase [24].

3-(2-Thiopnenyl)-1,4-dihydroindeno[1,2-*c*]pyrazole **18** (Figure **4**) was identified by high-throughput screening as a reversible ATP-competitive inhibitor ($IC_{50}$ = 0.93 μM) of VRGFR-2 [25]. Replacement of the thiophenyl by a phenyl maintained the potency (**19**, $IC_{50}$ = 1.2 μM). Attachment a polar basic 4-methylpiperazylmethyl at the 6- (**20**, $IC_{50}$ = 0.7 μM) and 7-position (**21**, $IC_{50}$ = 0.6 μM) produced about equipotent compounds with a slight improvement of activity compared to the parent compound **19**. Replacing the 3-phenyl group in **20** with six-membered aromatic heterocycles or aliphatic residues proved to be detrimental. Only thiophenes, even in comparison with other five-membered aromatic heterocycles, were well tolerated. The most potent compound **22** had an $IC_{50}$ of 0.40 μM. Switching the basic side chain from the 6- into the 7-position boosted in potency (**23**, $IC_{50}$ = 0.18 μM). Extending the methylene tether to an ethyl group in **23** slightly gained potency ($IC_{50}$ = 0.143 μM). Opening of the 1-methylpiperazine ring of **23** or elimination of one of the basic nitrogens was unfavorable. Extension of the N-alkyl group was tolerated but did not result in any significant improvement. Reduction of the basicity of either nitrogen by amide formation decreased the potency.

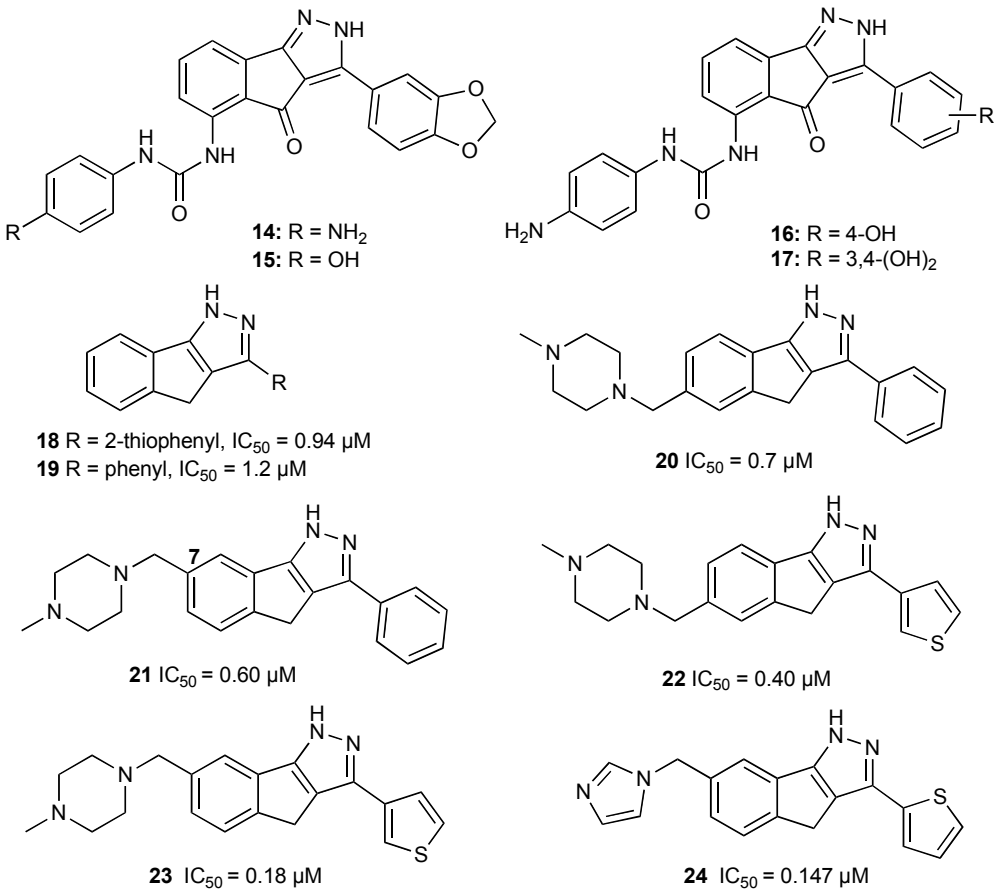



**Figure 4:** EGFR and/or VEGFR-2 inhibitors.

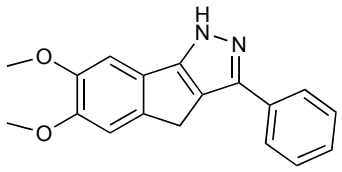
Replacement of the 1-methylpiperazine ring of **23** with a five-membered heterocycle was acceptable. The 1*H*-1,2,3-triazol substituted compound ($IC_{50}$ = 0.19 μM) almost retained the potency of **23**, while its 1*H*-1,2,4-triazol isomer had an improved *in vitro* activity ($IC_{50}$ = 0.108 μM). The imidazole-containing compound **24** ($IC_{50}$ = 0.147 μM) also showed good oral efficacy in an estradiol-induced murine uterine edema model of VEGF activity [25].

## 4. PLATELET-DERIVED GROWTH FACTOR RECEPTOR INHIBITORS

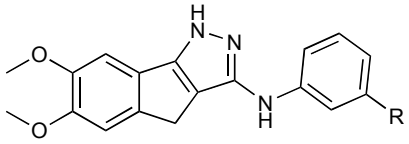
Angiogenesis plays an essential role in the physiology of neonatal growth, reproduction, and wound healing, while the pathophysiological angiogenesis contributes to sustain growth of solid tumors [26]. Platelet-derived growth factor receptors are cell surface tyrosine kinase receptors for members of the platelet-derived growth factor (PDGF) family. PDGFR is regard as an angiogenesis target and a driver of tumor cell development in some cancers. PDGFR can not only promote the overexpression of VEGF, another stimulator of angiogenesis, but also possess mitogenic and chemotactic effects. Therefore, PDGF pathway has the potential as a therapeutic strategy for inhibiting tumor-induced angiogenesis and growth [27, 28].

Compound **25** (Figure **5**) was identified through a compound library screening as a potent PDGFR-β kinase inhibitor ($IC_{50}$= 0.017 μM) with a modest tumor cell antiproliferative activity ($IC_{50}$<10 μM) [29]. Based on a homology model of the PDGF receptor kinase ATP binding site, the exchange of the 3-phenyl in **25** for a 3-aminophenyl group would benefit from the additional hydrogen bonding interaction with the hinge region. Compound **26** preserved the PDGFR kinase inhibition activity ($IC_{50}$ = 0.009 μM). Removal of the 6,7-dimethoxy in **26** led to much reduced potency (35-fold). The 7-methoxy and 5-methoxy derivatives of **26** showed slightly decreased activity, with $IC_{50}$ value of 0.018 and 0.054 μM, respectively, while the 6-methoxy analogue was much less active ($IC_{50}$ = 0.217 μM).

In a series of 6,7-dimethoxy analogues, introduction of a chloro at the *meta*-position of the phenylamine substituent was preferred to the *ortho*- or *para*-position. The 3-chloro compound **27** had an $IC_{50}$ of 0.003 μM, the 3-F (**28**), and 3-Br (**29**) derivatives exhibited similar



**25** $IC_{50}$ = 0.017 μM



**26** R = H, $IC_{50}$ = 0.009 μM
**27** R = Cl, $IC_{50}$ = 0.003 μM
**28** R = F, $IC_{50}$ = 0.0042 μM
**29** R = Br, $IC_{50}$ = 0.005 μM

**Figure 5:** PDGFR inhibitors.

potency, while the 3-Me ($IC_{50}$ = 0.012 μM), 3-MeO ($IC_{50}$ = 0.010 μM), 3-$CF_3$ ($IC_{50}$ = 0.038 μM), and 3-$CO_2CH_3$ ($IC_{50}$ = 0.033 μM) analogues maintained the potency. The 3-CN ($IC_{50}$ = 0.137 μM) and 3-$CO_2H$ ($IC_{50}$ > 0.8 μM) had much reduced activity. In addition, the dichlorophenyl analogues with substitution at the 2,5- and 2,4-positions were less active (in the micromolar range), while 3,4- and 3,5-dichlorophenyl substitution gave inhibitors with kinase $IC_{50}$ values of several hundred nanomolar [29].

Besides as an ATP competitive inhibitor of PDGFR-β kinase, compound **28** had good activity against PDGFR-α kinase ($IC_{50}$ = 0.045 μM) and the c-Abl kinase ($IC_{50}$ = 0.022 μM), had poor activity against the VEGFR ($IC_{50}$ = 3.1 μM) and bFGFR-1 kinases ($IC_{50}$= 45.8 μM), and was inactive against EGFR ($IC_{50}$ > 100 μM) and HER-2 ($IC_{50}$ > 10 μM) kinases. Compound **28** showed excellent tumor cell antiproliferative activity ($IC_{50}$ < 0.033 μM) in six of eight tumor cell lines. A cell counting experiment demonstrated that the antiproliferativ activity of **28** was cytostatic, not cytotoxic. Therefore, compound **28** represented a class of antitumor agents endowed with a dual mechanism of action: an antiangiogenic effect based upon the potent PDGFR-β kinase inhibition and a cytostatic effect, due to the potent and selective antiproliferative activity in human tumor cell lines [29].

## 5. CYCLIN-DEPENDENT KINASES INHIBITORS

Cyclin-dependent kinases (CDKs) are a family of protein kinases that are involved in cell cycle or transcription regulation. CDKs are activated by the binding to the cyclins to form specific complexes. Different CDKs are responsible for the cell cycle activation and cell cycle progression from G1 to mitosis [30]. Since most of the CDKs have been implicated in human cancers, an intense search has been undergoing for small molecule CDKs inhibitors as an approach to cancer chemotherapy.

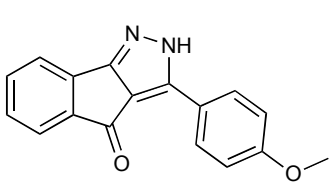
High throughput screening identified indenopyrazole **30** (Figure **10**) as a potent inhibitor of CDK4 and CDK2 with the $IC_{50}$ values of 45 and 26 μM, respectively. The pyrazole NH was important for the inhibitory activity, since it provided a key interaction with the enzyme. Further modifications at the indenopyrazole core were made [31, 32]. Enlarging the aromatic portion of the indenopyrazole core by a phenyl annulation at the 6- and 7-positions did not improve the activity, but the activity did not disappear entirely (CDK4, $IC_{50}$ = 80 μM). Dichlorination at the 6- and 7-positions of **30** led to decreased potency (CDK4, $IC_{50}$ = 55.5 μM). The insertion of a nitrogen at the 7-position (7-aza) maintained a similar activity against CDK4 ($IC_{50}$ = 34.5 μM), but increased (5-fold) the potency against CDK2 ($IC_{50}$ = 4.7 μM) as compared with **30**, however, the 8-aza derivative was completely inactive. In comparison with **30**, the introducing of a 5-hydroxy, 5-amino or 6-hydroxy group led to a modest increase (2-fold) in activity against CDK4, while a 6-amino group decreased the potency (2- to 10-fold) [32]. The 5-acetamide **31** was the first indenopyrazole analogue to break the micromolar potency with a CDK4 $IC_{50}$ of 460 nM and a CDK2 $IC_{50}$ of 510 nM. Small, linear substituents on the acetamide carbon of **31** were preferred. Branching at this α-carbon or direct substitution with an aromatic ring drastically reduced the potency [31]. Docking model indicated that compound **31** was buried deep in the ATP binding pocket and the two pyrazole nitrogens created key backbone hydrogen bonds with Val96. In addition, the 5-substituted acetamide carbonyl formed another key hydrogen bond with the side chain amino group of Lys35 [32].

A variety of phenacetyl derivatives, exemplified by **32**, showed similar affinity for CDK2 and CDK4 as compared to **32**, indicating that the binding pocket accommodating the phenacyl group was rather large and promiscuous, both lipophilic or hydrophilic and hydrogen bond donating or accepting substituents were tolerated. Replacement of the 4-aminophenyl group in

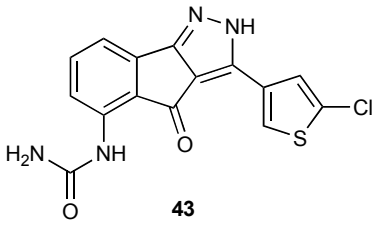
**32** by trisubstituted amines was generally preferred. The best analogues contained a six-membered saturated ring with an additional nitrogen at the 4-position (**33**). Extending hydrogen bond donating groups out from the 4-position of the six-membered ring further improved the potency, as exemplified by compounds **34−38** [32].

Replacing the acetamide methyl in **31** with a $sp^2$ hybridized nitrogen generated a sampling of ureas and semicarbazides [32]. The completely unsubstituted urea **39** was the most active. The introduction of one substituent (R) at the urea terminal nitrogen was considerably tolerated, while the disubstitued ureas were inactive. Introducing the semicarbazide moiety greatly enhanced the CDK4 activity, but the CDK2 was generally less affected in comparison with the corresponding glycinamide analogues. Three analogues, **40−42**, showed a 10-fold increase in activity against CDK4. The piperidine (R) and the pyrrolidine derivatives displayed similar potent activity towards CDK4 and CDK2 as the morpholine analogue **42**. Compound **42** was active against a transformed human colon cancer cell line (HCT116) with an $IC_{50}$ of 20 nM while maintaining an acceptable margin of activity against a normal fibroblast cell line, and a reasonable level of activity in the presence of human plasma proteins [32].

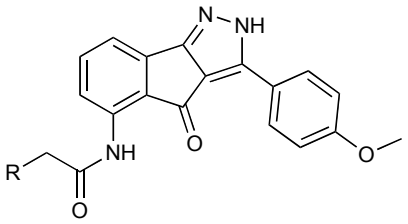
Taking the acetamide **31**, glycinamides (e.g. **33−38**), and the urea **39** as the leads, further modifications were made at the indenopyrazole C3-position [33]. Substitutions at the *para*-position of the phenyl ring at C3 were generally acceptable except the larger groups. When an alkyl was directly attached to C3, longer chain substituents were not tolerated; however, shorter alkyl groups and cyclic alkyls were acceptable. In general, heterocycles directly attached



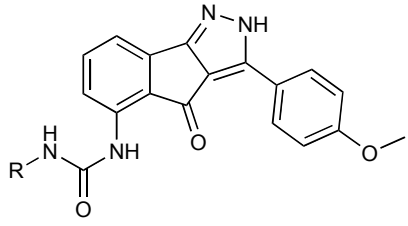
**30**



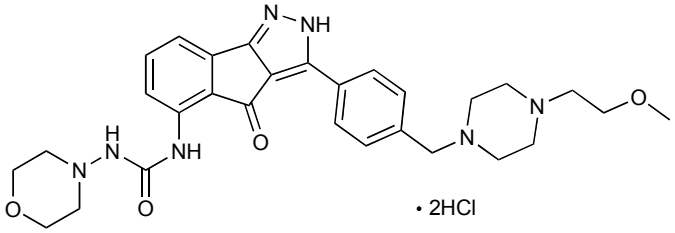
**43**



| | $IC_{50}$ (μM) | |
|---|---|---|
| | CDK4 | CDK2 |
| **31** R = H | 0.46 | 0.51 |
| **32** R = 4-$NH_2$Ph | 0.48 | 0.048 |
| **33** R = N-methylpiperazine | 0.125 | 0.012 |
| **34** R = 4-aminomethylpiperidine | 0.02 | 0.012 |
| **35** R = 4-amidopiperidine | 0.076 | 0.008 |
| **36** R = 4-hydroxymethylpiperidine | 0.082 | 0.011 |
| **37** R = 4-amidopiperazine | 0.064 | 0.008 |
| **38** R = 4-amidinopiperazine | 0.026 | 0.007 |



| | $IC_{50}$ (μM) | |
|---|---|---|
| | CDK4 | CDK2 |
| **39** R = H | 0.066 | 0.007 |
| **40** R = $Me_2NH$ | 0.021 | 0.005 |
| **41** R = 4-methylpiperazine | 0.009 | 0.012 |
| **42** R = morpholine | 0.012 | 0.008 |



**44** (RGB-286638)

**Figure 6:** CDK inhibitors.

to C3 were preferred and generated the most potent analogues. Compound **43** represented one of the more potent inhibitors that had $IC_{50}$ values of 57 and 13 nM against CDK4 and CDK2, respectively. It also showed good selectivity (>1000-fold) for CDK targets as compared with non-CDK targets. In addition, **43** was active in a variety of cancer cell lines. It showed 230-fold greater difference in activity against HCT116 ($IC_{50}$ = 130 nM) vs the normal human fibroblast AG1523. **43** blocked cell cycle G2/M progression in HCT116 cells. Moreover, CDK inhibition by **43** in HCT116 cells resulted in down-regulation of the CDK substrate Rb phosphorylation and activation of the apoptotic machinery by the cleavage of PARP and caspase-3 [33].

A semicarbazide indenopyrazole **44** (RGB-286638) with a substitution at the *para*-position of the phenyl ring at C3 was a potent inhibitor of CDK 1, 2, 3, 4, 5 and 9 ($IC_{50}$ = 1−5 nM), as well as 44- and 55-fold less active against CDK 7 and 6, respectively [34]. Since P53 deletion is associated with a very poor prognosis in multiple myeloma (MM), RGB-286638 was examined in MM cell lines with wild-type (wt)-p53 and p53 mutant [35]. In MM cells, RGB-286638 demonstrated preferential inhibition of CDK9 over other targets. In human p53-wt (MM.1S, MM.1R and H929) and p53-mutant (U266, OPM1 and RPMI) MM cells, RGB-286638 demonstrated its efficiency with the half-maximally effective concentrations ($EC_{50}$) ranging between 20 and 70 nM at 48 h. RGB-286638 also showed the same (50 nM) activity in freshly isolated tumor cells from MM patients. RGB-286638 induced caspase-dependent apoptosis in both wt-p53 and mutant-p53 cells through the down-regulation of RNA polymerase II phosphorylation and inhibition of transcription. RGB-286638 triggered p53 nuclear accumulation associated with increased p53 DNA-binding activity. In p53-knockdown and p53-mutant cells, RGB-286638 mediated p53-independent activity. In severe combined immunodeficient mice xenografted MM.1S cells, five days treatment (i.v.) with RGB-286638 with a dose of 30 mg/kg significantly suppressed MM tumor growth, with maximum tumor growth inhibition of 85% [35]. This study provided the rationale for the development of CDKIs as anti-MM agents.

## 6. TUBULINPOLYMERIZATION INHIBITORS

Microtubules, formed by α-and β-tubulin heterodimers, are essential components of the cytoskeleton and are involved in a number of important structural and regulatory functions, including the maintenance of cell shape and intracellular transport machinery as well as cell growth and mitosis. The rapid growth of cancer cells leads to their high dependence on tubulin polymerization/depolymerization, which makes tubulin a good target for anticancer drug developments [36−38].

A series of synthesized indenopyrazoles were tested for antiproliferative activity toward human cancer cell lines, HeLa (human cervical carcinoma), PC3 (human prostate cancer), and HCT116 (human colon cancer), and a normal cell line, HEK293 (human embryonic kidney) [39]. The results indicated that an alkoxy group at the C6-position was essential for the antiproliferative activity. The 6-methoxy analogue **45** (Figure **7**) and the 6-ethoxy derivative **46** exhibited very potent antiproliferative activity with $IC_{50}$ values between 8.9 and 35.6 nM, 20.4 and 60.2 nM, respectively. Introducing a hydroxyl or methoxy group at the 2-position of the aniline ring of **45** significantly reduced the antiproliferative potency. Introducing a hydroxyl, a methoxy, or a methoxycarbonyl group at the 3-position of aniline generated potent antiproliferative compounds, while a carboxylic acid group at the same position was not tolerated. In addition, a bulky isobutoxy at the 3-position of aniline slightly reduced the activity. A methoxy group at the 4-position of aniline was also not preferred. The 3,5-dimethoxyaniline analogue displayed increased potency, while the 3,4-dimethoxyaniline derivative was not active. The methyl ester **47** was the most potent compounds in this series with $IC_{50}$ values of 2.47, 2.64, 2.7, and 2.2 nM, toward Hela, PC3, HCT116, and HEK293 cells, respectively. Compound **47** inhibited tubulin polymerization in a concentration-dependent manner in a range of 3−30 μM. Furthermore, **47** inhibited microtubule formation and the acetylated tubulin accumulation, arrested cell cycle at G2/M stage in Hella cells, similar to the known tubulin polymerization inhibitor colchicine [39].

Based on the X-ray crystal structures of tubulin colchicine binding domain, our group designed a series of 1-methyl-indenopyrazoles with 7-oxoacetamides of 4- to 8-atom lengths as potential tubulin polymerization inhibitors [40]. In the growth inhibitory assay against four human cancer cell lines, HepG2 (human liver carcinoma), Hela (human cervical carcinoma), PC3 (human prostate cancinoma), and MCF-7 (human breast adenocarcinoma), the acetamide **48** was just as potent as colchicine and paclitaxel, with $IC_{50}$ values ranging from 7.4 to 32.3 nM. The amide N-methylation in **48** led to 5.5- to 27-fold decrease in potency, while

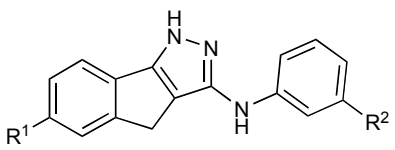
N-dimethylation further deteriorated its activity. Replacing the N-methyl by an ethyl group also led to reduced potency. Further introducing a hydroxyl, an amino, a dimethylamino, or a methoxy group at the β-position of ethyl substituent improved the potency of the corresponding N-ethyl analogue toward some cancer cell lines in different degrees, but a cyano group at the same position was not tolerated. The N-cyclopropylacetamide maintained considerable potency ($IC_{50}$ = 0.65−3.07 μM), while the N-cyclopropylmethyl-acetamide with one carbon elongation displayed much reduced activity. Introducing an unsaturated 2-propnyl or allyl substituent at the acetamide nitrogen was considerable tolerated. The N-hydroxyacetamide **49** exhibited potency comparable to that of **48** with $IC_{50}$ values ranging from 13.2 to 33.5 nM, but the hydroxyl methylation of **49** greatly reduced its activity [40]. Therefore, the substituents at the acetamide nitrogen affected their tumor cell growth inhibitory potency. The 7-oxoacetamides with a 4- or 5-atom length gave the best results, possibly due to the limited distance and space at the interface between the α- and β-subunit of tubulin heterodimers. In addition, a hydrogen-bond-donor/-acceptor atom (O, N) at or near the terminal of the substituents was preferred for the better antiproliferative activity.

Both compounds **48** and **49** competed with colchicine in binding to tubulin and inhibited tubulin polymerization in a concentration-dependent way, with calculated $IC_{50}$ values of 4.62 μM and 5.33 μM, respectively. In A549 cells, **48**was observed to disorganize microtubule and arrest cell cycle in G2/M phase through the alterations in the expression of cyclin B1 and p-cdc2, induce cell apoptosis through the activation of caspase-3 and PARP. Moreover, **48** could inhibit capillary tube formation and has the potential as a vascular disrupting agent. In non-small cell lung cancer xenografts mouse model, **48** demonstrated its potential as anticancer agent by suppressing tumor growth (59.1%) at a dose of 50 mg/kg (i.p.) without obvious toxicity. Molecular docking suggested the acetamide substituent at the 7-position might be responsible for the high potency of **48** by occupying an additional open cavity up to the α-tubulin subunit in the interfacial region and by forming critical hydrogen bonding with Serα178 at the interfacial surface [40].

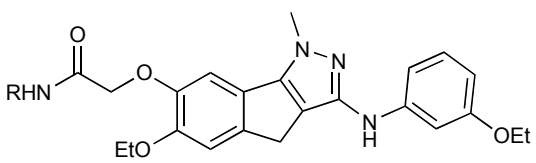
## 7. HYPOXIA-INDUCIBLE FACTOR 1 INHIBITORS

Hypoxia-inducible factor-1 (HIF-1) is a heterodimeric basic helix-loop-helix transcription factor composed of the oxygen-regulated HIF-1α and the constitutively expressed HIF-1β subunits. The activated HIF is associated with a variety of pathological conditions, including inflammation and cancer. HIF-1α is overexpressed in many human cancers, including brain, breast, colon, lung, ovary, and prostate. HIF-1 controls gene expression influencing tumor angiogenesis, metastasis, and invasion [41, 42]. Therefore, HIF-1 becomes a feasible and promising target for anticancer drug developments.

In a series of 3-aniline substituted indenopyrazoles, the compound with no substituents at both the aniline and indenopyrazole phenyl rings was not active. When the 3-aniline was unsubstituted, introducing one methoxy group at either the indenopyrazole 5-, 6-, 7-, 8-position, or two substituents at the 5,6-positions resulted in weak inhibitory activity [43]. However, when there were no substituents at the indenopyrazole phenyl ring, the substitution at the 3-aniline ring led to potent HIF-1 inhibitors. The indenopyrazole derivatives having one methoxy group at the 3- or 4-position, and dimethoxy groups at the 3,4-position of the aniline ring inhibited the hypoxia induced HIF-1 transcriptional activity with $IC_{50}$ values of 1.4, 6.1, and 3.2 μM, respectively. A cyclic ether skeleton on aniline ring dramatically enhanced the potency. The 3,4-methylenedioxyaniline analogue inhibited the HIF-1 transcriptional activity with an $IC_{50}$ of 0.27 μM, while the 3,4-ethylenedioxyaniline derivative **50** (Figure **8**) was the most potent ($IC_{50}$ = 0.014 μM). The methoxy group substitution at 5-position of **50** decreased the inhibitory effect on HIF-1 transcriptional activity ($IC_{50}$ = 0.034



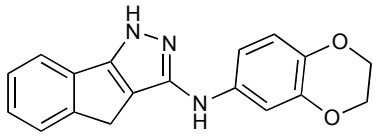

**45** $R^1$ = MeO, $R^2$ = H
**46** $R^1$ = EtO, $R^2$ = H
**47** $R^1$ = MeO, $R^2$ = $CO_2Me$




**48** R = H
**49** R = OH

**Figure 7:** Tubulin polymerization inhibitors.

μM). The antiproliferative activities of indenopyrazoles with substituted 3-anlines were weaker than their HIF-1α transcription inhibitory activities by 2 orders of magnitude, indicating that these indenopyrazoles suppressed HIF-1 transcriptional activity without affecting cell viability. In addition, compound **50** did not affect both HIF-1α protein accumulation and HIF-1α/HIF-1β heterodimerization in nuclei under the hypoxic conditions, suggesting that **50** probably affected the transcriptional pathway induced by the HIF-1α/HIF-1β heterodimer [43].



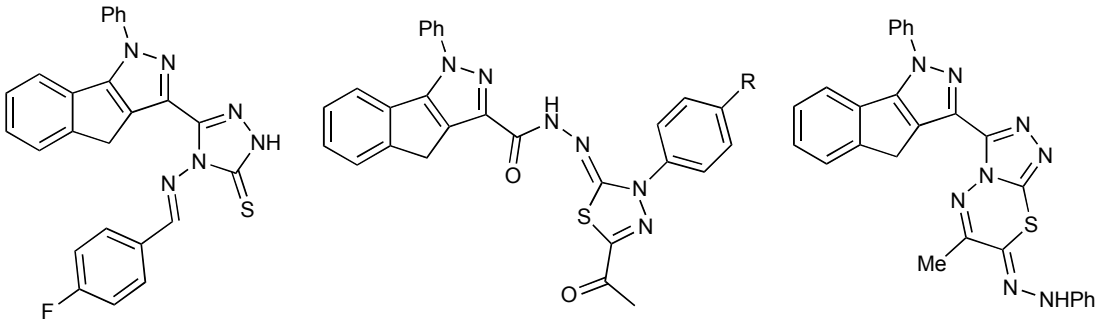
**50**

**Figure 8:** HIF-1 inhibitors.

## 8. OTHERS

A series of 1,3-thiazole, 1,3,4-thiadiazole, 1,2,4-triazole, and 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine were attached to the 1,4-dihydro-1-phenylindeno[1,2-*c*]pyrazole moiety at the 3-position and were evaluated for their protective activities against DNA damage induced by bleomycin–iron assay. The Schiff base **51** derived from the 4-amino-1,2,4-triazole-5-thione, the 1,3,4-thiadiazole derivatives **52** and **53**, and the 1,2,4-triazolo[3,4-b]-1,3,4-thiadiazine analogue **54** (Figure **9**) exhibited high protection activity against DNA damage, while all the others showed low to modest activity [44].

A series of 3-(4-chlorophenyl)-[1,2-*c*]pyrazol(in)es substituted with benzenesulfonamide, N,N-disubstituted sulfonylurea, sulfonylthiourea pharmacophores, and some derived thiazolidinone and thiazoline ring systems were evaluated against a panel of 60 different tumor cell lines [45]. The parameter $GI_{50}$ (growth inhibitory activity) represents the concentration of the compounds causing 50% decrease in net cell growth, the TGI value (cytostatic activity) corresponds to the concentration of the compounds resulting in total growth inhibition, and the $LC_{50}$ value (cytotoxic activity) is the concentration of the compounds causing net 50% loss of initial cells at the end of the incubation period (48 h). The indeno[1,2-*c*]pyrazoline sulfonamide **55** (Figure **10**) exhibited a broad spectrum antitumor activity with its MG-MID (the average sensitivity of all cell lines toward the test agent) values for the $GI_{50}$, TGI, and $LC_{50}$ of 9.12, 34.7, and 83.2 μM, respectively. The oxidized pyrazole **56** was the most active with the highest cytostatic (TGI = 33.1 μM) and cytotxic potentials (MG-MID $LC_{50}$ = 66.1 μM). **56** displayed special effectiveness on the leukemia subpanel with the $GI_{50}$, TGI, and $LC_{50}$ (MG-MID) levels of 5.93, 19.1, and 59.2 μM, respectively. The disubstituted sulfonylureido derivative **57** exhibited remarkable antitumor activity. It was just as potent as **55** in the $GI_{50}$ and TGI (MG-MID) levels (9.12 vs 9.12 and 33.9 vs 34.7 μM, respectively), and showed slightly improved cytotoxic activity (MG-MID $LC_{50}$ 77.6 vs 83.2 μM). However, the replacement of the cyclohexyl (R) group in **57** by a phenyl or 1-naphthyl led to the loss of activity. In the oxidized pyrazole series, the substituent in the sulfonylureido functionality influenced the antitumor activity. The cyclohexyl moiety in compound **58** was the most favorable substituent with $GI_{50}$, TGI, and $LC_{50}$ (MG-MID) values of 10.2, 34.7, and 81.3 μM, respectively. The phenyl (R) substituted analogue **59** displayed a marked reduced potency in the antitumor activity to the half at the $GI_{50}$ and TGI levels (19.5 and 63.1 μM, respectively) and cytotoxic effect (MG-MID $LC_{50}$ = 97.7 μM). Replacement of the cyclohexyl group in **58** by 1-naphthyl with the increased size and aromaticity abolished the antitumor activity. In comparison with its parent pyrazoline **57**, compound **58** had almost the same antitumor profile except for the activity against leukemia and breast cancer subpanels



**51** **52** R = H, **53** R = F **54**

**Figure 9:** Indenopyrazole derivatives against DNA damage.

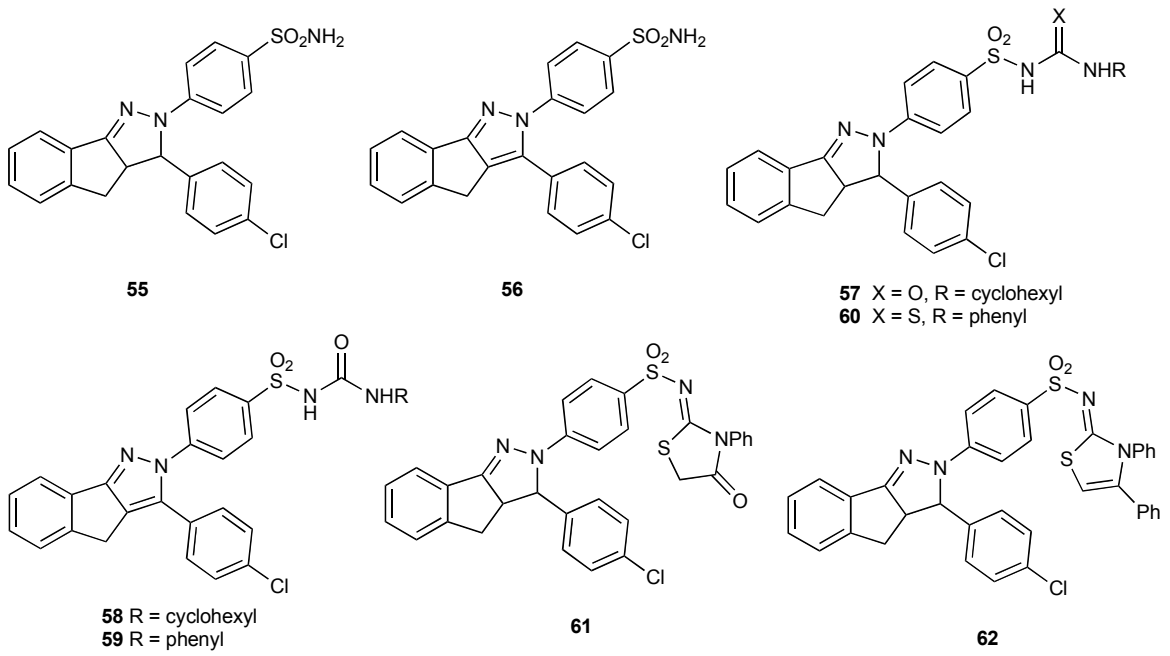



**Figure 10:** Indenopyrazole derivatives as antiproliferative agents.

which showed about 2-fold improvement in activity at the $GI_{50}$ level (2.50 vs 5.73 and 7.25 vs 12.7 μM, respectively). Conversion of the sulfonamide **55** to the substituted sulfonylthioureido derivatives generated only one active compound **60** (X = S, R = phenyl) with a significant reduction (2- to 3-fold) in the overall antitumor activity when compared with the parent sulfonamide **55** and the sulfonylureido isostere **58**. In addition, incorporation of the thioureido moiety partly in a rigid structure led to two active compounds, the 2-oxothiazolidine **61** and thiazoline **62**, that showed a significant reduction in the growth inhibitory, cytostatic potencies and a complete loss of the cytotoxic activity, though the oxothiazolidine **61** was relatively more active with $GI_{50}$ and TGI (MG-MID) 30.2 and 77.6 μM, respectively, when compared with the thiazoline **62** ($GI_{50}$ and MG-MID TGI (MG-MID of 41.7 and 91.2 μM, respectively) [45]. In summary, in comparison with the parent pyrazoline analogues, the oxidized pyrazoles had better antitumor activity, whereas the benzenesulfonamides and the N,N-disubstituted sulfonylureas showed significant better antitumor spectrum than the sulfonylthioureido and the derived thiazole derivatives.

## CONCLUSION

As a privileged structure, indenopyrazole framework is widely used in the design of anticancer agents. By varying substituents on different positions of the indenopyrazole core, potent and selective inhibitors targeting a variety of biological proteins can be obtained. Despite the current progresses, there in general lacks the deep studies of the *in vivo* anticancer efficiency, the drug-like and pharmacokinetic properties. There is also the possibility that some indenopyrazoles might display their *in vitro* anticancer activity by acting on a multiple of biological targets. Therefore, future studies should pay more attention for the target selectivity.

## LIST OF ABBREVIATIONS

CHK = Checkpoint kinase

EGFR = Epidermal growth factor receptor

VEGF = Vascular endothelial growth factor

KDR = Kinase insert domain receptor

PDGFR = Platelet-derived growth factor receptor

CDK = Cyclin-dependent kinase

HIF-1 = Hypoxia-inducible factor 1

SAR = structure−activity relationship

PARP = poly-ADP-ribose polymerase

## CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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