Novel indazole derivatives as potent apoptotic antiproliferative agents by multi-targeted mechanism: Synthesis and biological evaluation

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A B S T R A C T

Indazole is a significant class of heterocyclic compounds with a wide range of biological activity. We display here the synthesis and biological evaluation of a novel series of indazole derivatives **6a-v**, which are differently substituted at the 6-position of the indazole moiety. The antiproliferative activity of compounds **6a-v** was tested against four human cancer cell lines, using the MTT assay and doxorubicin as the reference drug. Compounds **6f, 6i, 6j, 6 s, and 6n** were the most effective synthesized derivatives, with GI_{50} values of 0.77, 0.86, 1.05, 1.05, and 1.07 µM, respectively, against the 4 cell lines, in comparison to the control doxorubicin (GI_{50} = 1.10 µM). Compounds **6f, 6i, 6j**, and **6 s** the most potent derivatives as antiproliferative agents, displayed the utmost inhibitory activity against EGFR, and CDK2 and c-Met. Compounds **6f, 6n, and 6 s** induced apoptosis through cytochrome *C* overexpression and activation of the intrinsic apoptotic pathway generated by the investigated compounds.

1. Introduction

The cancer is one of the major diseases that cause death world-wide; thus, the world has been paying close attention to its treatment. In comparison to biological therapy, radiotherapy, and chemotherapy continue the basis of modern treatment. Nonetheless, the wide range of these suggested medications are constrained by a bound therapeutic index and commonly obtained resistance. As a result, the expansion of innovative anticancer medicines with minimal toxicity and significant efficacy remains critical [1,2].

Recently, a single-aim treatment strategy has been extensively documented that leads to chemotherapy resistance [3]. This issue addressed by combination treatment has been approved for clinical usage [4]. Although combination therapy has the potential for additional and even synergistic advantages, it frequently outcome unforeseen adverse effects such as higher toxicity. Dual or multiple target medications having a minimum risk of drug interactions. Further, the alternate options for combination therapy are the enhanced pharmacokinetics (PK), and safety profiles. A dual or multiple target kinase also assist to avoid poor patient adherence, negative off-target effects, drug interactions, and high manufacturing costs [5].

An indazole motif can be used as both a functional substituent and a scaffold for small molecule medicines. Bioactive compounds containing indazole have pharmacological effects on a variety of disorders [6–8], including infectious, inflammatory, neurodegenerative and cancer diseases [9–11]. Some indazole compounds discovered as kinase inhibitors, in particular, have strong anti-cancer action *in vitro* and *in vivo*. Pazopanib **I** (multi-kinase inhibitor), merestinib **II** (c-Met inhibitor), entrectinib **III** and axitinib **IV** (multi-kinase-inhibitors) are indazolebased compounds approved for the treatment of different cancer types [12–19]. Furthermore, **Y2874455 V** and **CFI-400945 VI** are two indazole-based kinase inhibitors investigated in clinical trials in form of

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combination therapy for treatment of acute myeloid leukemia and breast cancer [20–26], Fig. 1.

Moreover, compound **VII** (Fig. 1) is an indazole inhibitor of several kinases; however, not only did it have an immense impact on NSCLCrelated kinases, but it also had a big impact on NCI-H1581 NSCLC xenografts (TGI = 66.1%) and a promising pharmacokinetic profile.

Compound **VIII**, an indazole-based derivative, demonstrated significant EGFR inhibitory activity with IC_{50} values of 0.50, 0.07, and 1.70 µM for L858R, L858R/T790M, and wild-type EGFR, respectively. This analogue, in particular, demonstrated a significantly higher inhibitory impact on the drug-resistant mutation of EGFR [27].

Recently, we identified compounds **IX-XI** (Fig. 2) as dual-targeted kinase inhibitors with anticipative antiproliferative activity [28–30]. Compounds **IX** and **X** showed promising dual inhibition of EGFR and BRAF, while compound **XI** inhibited both BRAF and p38α. Compound **XI** inhibited proliferation in the NCI-60 5-dose experiment, with GI_{50} values less than 2 µM against 33 cell lines, essentially for ovarian IGROV1 cells ($GI_{50} = 0.46$ µM), leukemia SR cells ($GI_{50} = 0.28$ µM), renal ACHN cells ($GI_{50} = 0.38$ µM), and melanoma LOX IMV1 cells ($GI_{50} = 0.17$ µM).

Given the promising properties of indazole derivatives as potent anticancer agents, and as a follow-up to our work on the synthesis of dual or multi-targeted kinase inhibitors, we display here the synthesis and biological valuation of a recent series of indazole derivatives **6a-v** (Fig. 2), which are differently substituted at the 6-position of the indazole moiety. The novel compounds have been tested for antiproliferative activity against a panel of cancer cell lines *in vitro.* Furthermore, the most active compounds were evaluated against EGFR, c-Met, and CDK2 TKs as prospective targets for mechanistic action (These targets were chosen based on published outcomes for indazole derivatives **I-VIII**).

2. Results and discussion

2.1. Chemistry

Synthesis of compounds **6a-v** was outlined in Scheme 1. Aryl nitriles **2a–v** were synthesized by adding aqueous ammonia solution to aldehydes **1a–v** in THF, followed by sublimed iodine, and stirring the

Fig. 1. Structure of indazole-based compounds as kinase inhibitors.

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reaction mixture for 3 h [31]. Without additional purification processes directly, aryl nitrile **2a-v** is added to the mixture of Hydroxylamine Hydrochloride in MeOH as the solvent, with anhydrous $NaHCO_3$ as the basic catalyst, for 30 min, and then the mixture is refluxed for 5–8 h to obtain amidoximes **3a-v** [32]. 1*H*-indazole-6-carboxylic acid **5** was obtained from hydrolyzing methyl 1*H*-indazole-6-carboxylate **4** with NaOH in MeOH to give carboxylic acid in quantitative yield [33]. At room temperature, the esterification reaction takes place by stirring a mixture of the 1*H*-indazole-6-carboxylic acid in DMF, adding EDC and HOAt as coupling agents. The reaction mixture was stirred for 30 min at room temperature; thereafter, the amidoximes **3a-v** were added. For 24 h, the mixture was stirred at room temperature to ensure that the reaction had completed. Following extraction, the formed products were purified by column chromatography, yielding compounds **6a-v** with 50–70% yields.

Reagent and reaction conditions: i) NH_3, THF, I_2, r.t. 3 h, 65–80; ii) NH_2OH, methanol, $NaHCO_3$, reflux 3–5 h, 80–90%; iii) NaOH, MeOH, r. t. 1 h.; iv) EDCI, DMF, HOAT, r.t. 24 h.

Comp. No. R Comp. No. R

1a, 2a, 3a,
and **6a**
1i, 2i, 3i,
and **6i**
1b, 2b, 3b,
and **6b**
1j, 2j, 3j,
and **6j**
1c, 2c, 3c,
and **6c**
**1 k, 2 k, 3
k,** and **6 k**
1d, 2d, 3d,

and **6d**
1 l, 2 l, 3 l,
and **6 l**
1e, 2e, 3e,
and **6e**
1 m, 2 m, 3
m, and 6 *m*

Fig. 2. Structure of compounds **IX-XI** and new indazole-based compounds **6a-v.**

Scheme 1. Synthesis of compounds **6a-v.**

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(*continued*)

Comp. No. R Comp. No. R

1f, 2f, 3f,
and **6f**
1n, 2n, 3n,
and **6***n*
1 g, 2 g, 3
g, and 6
g
1o, 2o, 3o,
and **6o**
1 h, 2 h, 3
h, and 6
h
1p, 2p, 3p,
and **6p**
1q, 2q, 3q,
and **6q**
1r, 2r, 3r,
and **6r**
1 s, 2 s, 3 s,
and **6 s**
1u, 2u, 3u,
and **6u**
1 t, 2 t, 3 t,
and **6 t**
1v, 2v, 3v,
and **6v**

The structure of novel compounds **6a-v** was determined using boiling point, mass spectral analysis and NMR spectroscopy. According to ^1H and ^{13}C NMR spectra, the products were distinguished by the presence of extra peaks not present in the carboxylic acid starting material **5**. In the ^1H NMR spectrum of **6 s**, the absence of the carboxylic acid OH signal and the appearance of two sets of doublets at 7.74 and 7.68 ppm suggest aromatic *p*-substitution. The spectrum also revealed the acetamido group's distinctive signals in the form of a singlet signal of three protons at 2.08 ppm of methyl protons (NHCOCH$_3$) and a singlet signal of one proton at 10.16 ppm (NHCOCH$_3$). The spectrum also showed a singlet signal of the NH$_2$ group (2H) at 6.93 ppm, a singlet signal of the NH group of indazole (1H), and aromatic protons. The ^{13}C NMR spectrum of **6 s** revealed two signals at 168.62 and 164.09 ppm corresponding to two carbonyl groups (NHCO and COO), four additional carbon signals in the aromatic, and a methyl carbon signal at 24.05 ppm (CH$_3$CO). HRMS yielded a peak for [M + Na]$_+$ at *m/z* 360.1073 that was compatible with the chemical formula $C_{17}H_{15}N_5NaO_3$.

2.2. Biology

2.2.1. In vitro antiproliferative assay

2.2.1.1. Cell viability assay. In the cell viability experiment [34], the human mammary gland epithelial cell line (MCF-10A) was used. The cell viability of compounds **6a-v** was tested using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay after 4 days of culture with MCF-10A cells [35]. There were no cytotoxic effects encountered in any of the compounds tested, and the majority of the compounds tested at 50 µM had cell viability of more than 85%.

2.2.1.2. Antiproliferative assay. The antiproliferative activity of compounds **6a-v** was tested against four human cancer cell lines, including HT-29 (colon cancer cell line), A-549 (epithelial cancer cell line), Panc-1 (pancreas cancer cell line), and MCF-7 (breast cancer cell line), using the MTT assay [36,37] and doxorubicin as the reference drug. Further, the median inhibitory concentration (IC$_{50}$) was calculated for each compound. The findings are shown in Table 1.

Compounds **6f, 6i, 6j, 6 s, and 6n** (R = 3,5-dimethoxyphenyl, 2-

Table 1

IC$_{50}$s of antiproliferative assay of compounds **6a-v** and Doxorubicin.

Comp. Cell

	viability %	Antiproliferative activity IC$_{50}$ ± SEM (µM)				
		A-549	MCF-7	Panc-1	HT-29	Average
6a	85	1.50 ± 0.50	1.90 ± 0.50	1.70 ± 0.40	1.10 ± 0.10	1.55
6b	86	3.20 ± 0.50	3.40 ± 0.40	3.20 ± 0.20	3.10 ± 0.20	3.25
6c	87	3.90 ± 0.50	3.10 ± 0.40	3.70 ± 0.70	3.70 ± 0.50	3.60
6d	91	4.90 ± 0.70	4.70 ± 0.40	4.70 ± 0.40	4.10 ± 0.20	4.60
6e	87	3.90 ± 0.60	3.70 ± 0.40	3.10 ± 0.30	3.20 ± 0.30	3.50
6f	89	0.80 ± 0.10	0.60 ± 0.10	0.80 ± 0.10	0.90 ± 0.10	0.77
6 g	91	1.60 ± 0.40	1.30 ± 0.30	1.25 ± 0.30	1.60 ± 0.50	1.45
6 h	85	2.30 ± 0.40	2.10 ± 0.30			

2.75
± 0.40
2.80
±
0.40
2.50
6i 90 0.90
±
0.10
0.80
± 0.10
0.80
± 0.10
0.95
±
0.20
0.86
6j 87 1.20
±
0.20
0.90
± 0.20
1.00
± 0.30
1.10
±
0.20
1.05
6 k 90 1.40
±
0.40
1.10
± 0.30
1.70
± 0.40
1.10
±
0.20
1.30
6 l 89 7.20
±
1.00
6.90
± 1.00
7.50
± 1.00
7.10
±
1.20
7.20
6 *m* 87 2.90
±
0.50
2.10
± 0.40
2.70
± 0.70
2.70
±
0.50
2.60
6*n* 91 1.20
±
0.20
1.00
± 0.30
1.00
± 0.20
1.10
±
0.20
1.07
6o 86 5.90
±
0.70
5.70
± 0.60
6.10
± 0.70
6.15
±
0.70
6.00
6p 87 5.10
±
0.60

Compound		Value 1	Value 2	Value 3	Value 4	Value 5
		4.90 ± 0.50	5.20 ± 0.60	5.30 ± 0.40	5.15	
6q	89	1.80 ± 0.40	1.50 ± 0.30	1.40 ± 0.40	2.10 ± 0.50	1.70
6r	90	1.90 ± 0.40	1.60 ± 0.30	1.90 ± 0.40	2.20 ± 0.40	1.90
6 s	91	1.10 ± 0.20	1.00 ± 0.30	1.05 ± 0.20	1.10 ± 0.20	1.05
6 t	89	5.40 ± 0.60	5.20 ± 0.50	5.50 ± 0.70	5.60 ± 0.70	5.40
6u	86	4.30 ± 0.60	4.00 ± 0.50	4.20 ± 0.60	4.10 ± 0.60	4.15
6v	89	7.90 ± 1.20	7.80 ± 1.00	8.30 ± 1.20	8.20 ± 1.20	8.05
Doxorubicin	–	1.40 ± 0.58	0.90 ± 0.62	1.00 ± 0.82	1.20 ± 0.80	1.10

naphthyl, 4-chlorophenyl, 4-acetamidophenyl, and 4-methoxy-3-pyridyl, respectively) were the most effective synthesized derivatives, with GI_{50} values of 0.77, 0.86, 1.05, 1.05, and 1.07 µM, respectively, against the 4 cell lines, in comparison to the control doxorubicin (GI_{50} = 1.10 µM). These five derivatives inhibited A-549 and HT-29 cancer cell lines more effectively than doxorubicin.

The 3,5-dimethoxyphenyl derivative **6f** (R = 3,4-dimethoxyphenyl) was the most effective of all the synthesized derivatives against the four cell lines, with a GI_{50} value of 0.77 µM, in relative to the control doxorubicin (GI_{50} = 1.10 µM) and was found to be more potent than doxorubicin against the four tested cell lines. The unsubstituted derivative **6q** (R = phenyl) was about 2 times less potent than **6f**, with a GI_{50} value of 1.70 µM. Moreover, the antiproliferative action was reduced when the 3,4-dimethoxyphenyl moiety in **6f** was replaced by 4-pyridine, 2-hydroxymethyl furan, 2-pyridine, 3-thiophene, pyrazole, 1-oxo-1,3-dihydroisobenzofuran, and furan in compounds **6b-e**, **6o**, **6p**, and **6 t**, respectively, pointing to the fact that the pattern of substitution and/or the position of the phenyl moiety are critical for antiproliferative activity.

Compound **6i** (R = 2-naphthyl) was the second most potent derivative, with a GI_{50} value of 0.86 µM against the four cell lines, in comparison to doxorubicin (GI_{50} = 1.10 µM), and it was more effective than doxorubicin against all cancer cell lines examined. The 1-naphthyl derivative **6v** (R = 1-naphthyl) was almost ten times less active than **6i**, with a GI_{50} = 8.05 µM.

Compound **6j** (R = 4-chlorophenyl) ranks third in terms of activity behind compounds **6f** and **6i**, with a GI_{50} of 1.05 µM, but it is still more active than doxorubicin (GI_{50} = 1.10 µM), also investigated substituting halogen atoms for the 4-chlorophenyl in 6j. The findings showed that replacement of the chlorine atom in compound **6j** with bromine, fluorine, and iodine atoms as in compounds **6 k**, **6 l**, and **6 m**, respectively, resulted in a decrease in antiproliferative activity, with the 4-fluorophenyl derivative, **6 l**, being the least active with a GI_{50} of 7.20 µM, which is approximately 7-fold less active than **6j**.

2.2.2. Multi-kinase (CDK-2, EGFR, and c-Met) inhibitory assays

2.2.2.1. CDK2 inhibitory assay. Cyclin-dependent kinase 2 (CDK2) regulates cell cycle progression into the S- and M☐ phases. CDK2 activity is mainly unnecessary for normal development, but it is strongly linked to tumor growth in a variety of cancers. Although the significance of CDK2 in carcinogenesis has been debated, new evidence suggests that selective CDK2 inhibition may provide a therapeutic benefit against some malignancies, and it remains appealing as an approach to pursue in anticancer drug development. A number of small-molecule CDK2 inhibitors have advanced to clinical studies. A CDK2-selective inhibitor, on the other hand, has yet to be found [38].

The CDK2 potential inhibitory activity of compounds **6a, 6f, 6 g, 6i, 6j, 6 k, 6n,** and **6 s** was investigated [39]. In Table 2**,** the IC_{50} values show that all compounds inhibited CDK2 with IC_{50} values ranging from 14 nM to 27 nM, with compounds **6f, 6i**, and **6j** (IC_{50} = 14, 16, and 19 nM, respectively), which were more potent than the control dinaciclib (IC_{50} = 20 nM). Compounds **6a, 6 g, 6 k, 6n,** and **6 s** demonstrated good anti-CDK2 activity with IC_{50} values of 30, 27, 25, 22, and 21 nM, respectively. Compounds **6f, 6i, 6j**, and **6 s** the most potent derivatives as antiproliferative agents, displayed the utmost inhibitory activity against CDK2. The outcomes of the assay indicate that these compounds may act as potential targets for CDK2.

2.2.2.2. EGFR inhibitory assay. Numerous previous studies demonstrated that various indazole-based derivatives are effective EGFR inhibitors [40,41]. Compounds **6a**, **6f**, **6 g**, **6i**, **6j**, **6 k**, **6n**, and **6 s** were further studied for their ability to inhibit the EGFR-TK enzyme [42]. Table 2 displays the IC_{50} values. The findings indicate that for each compounds inhibited EGFR with IC_{50} values extending from 80 nM to 275 nM. Throughout, the investigated compounds were at least 1.2-fold less potent than the reference erlotinib (IC_{50} = 70 nM). Compounds **6n** and **6 s** evidenced the most potent EGFR inhibitory activity with IC_{50} values of 80, and 85 nM respectively. Moreover, compounds **6f, 6i**, and **6j** the most potent derivatives as antiproliferative agents, exhibited moderate activity against EGFR (IC_{50} = 89, 130, and 275 nM).

2.2.2.3. c-Met inhibitory assay. Compounds **6a**, **6f**, **6 g**, **6i**, **6j**, **6 k**, **6n**, and **6 s** were evaluated [43] for their capacity to inhibit the c-Met enzyme based on the existing literature demonstrating the c-Met inhibitory efficacy of the indazole-based drug merestinib [13]. Table 2 displays the IC_{50} values. According to the findings, all compounds showed potent inhibition of c-Met with IC_{50} values extending from 3.10 nM to 6.30 nM in comparison to the reference foretinib (IC_{50} = 2.5 nM). Once again, compounds **6n** and **6 s** demonstrated the most potent c-Met inhibitory activity with IC_{50} values of 3.10, and 3.50 nM, respectively. On the other hand, compounds **6f, 6i,** and **6j** the most potent derivatives as antiproliferative agents, exhibited promising activity against c-Met (IC_{50} = 4.10, 5.50, and 6.30 nM).

Based on the outcomes of this study, we may infer that CDK2, EGFR, and c-Met are promising targets for compounds **6f, 6n,** and **6 s**. More indepth mechanistic exploration may be necessary in the future.

2.2.3. Apoptosis assay

Previous study has shown that indazole compounds can trigger apoptosis [44]. To determine the proapoptotic potential of our new compounds, the most active compounds (**6f, 6n,** and **6 s**) were examined for their capacity to initiate the apoptosis flow in the MCF-7 breast cancer cell line.

2.2.3.1. Activation of proteolytic caspases cascade. Caspases play a critical role in initiating and terminating the apoptotic process [45]. Caspase-3 is an important caspase that cleaves numerous proteins in cells, resulting in apoptotic cell death [46]. The effect of **6f**, **6n**, and **6 s** on caspase 3 was studied and compared to the reference drug doxorubicin. The results showed that the tested compounds increased the level of active caspase 3 by 7.7–9 folds when compared to control cells, and that **6f, 6n,** and **6 s** possessed remarkable overexpression of caspase-3 protein level (602.40 ± 4.5, 505.30 ± 4.0 and 510.50 ± 4.5 pg/mL, respectively) when compared to doxorubicin (503.2 ± 4.20 pg/mL). All of the compound examined exhibited a larger increase in active caspase 3 than the control doxorubicin, Fig. 3.

To further emphasize the role of intrinsic and extrinsic apoptotic pathways in the antiproliferative activity of compounds **6f, 6n,** and **6 s**, their effect on caspases 8 and 9 was also examined. The results indicate that compound **6f** increases caspase 8 and 9 levels by 11.5 and 20 folds, respectively, whereas compounds **6n** and **6 s** increase caspase 8 and 9 levels by 10.5–11 folds and 18–18.5 folds, respectively. Comparing with the control cells, the caspase 9 levels were increased because of the effect of activating both intrinsic and extrinsic pathways was more

Table 2

IC_{50}s of multi-kinase assays of compounds **6a**, **6f**, **6 g**, **6i**, **6j**, **6 k**, **6n**, and **6 s.**

Code No.	CDK2 IC_{50} ± SEM (nM)	EGFR IC_{50} ± SEM (nM)	c-Met IC_{50} ± SEM (nM)
6a	30 ± 3	100 ± 5	4.50 ± 0.30
6f	14 ± 1	89 ± 3	4.10 ± 0.10
6 g	27 ± 2	110 ± 5	5.10 ± 0.30
6i	16 ± 2	130 ± 5	5.50 ± 0.20
6j	19 ± 2	275 ± 10	6.30 ± 0.20
6 k	25 ± 2	145 ± 7	4.80 ± 0.20
6n	22 ± 2	80 ± 3	3.10 ± 0.20
6 s	21 ± 2	85 ± 6	3.50 ± 0.20
Dinaciclib	20 ± 2	ND	ND
Erlotinib	ND	70 ± 3	ND
Foretinib	ND	ND	2.5 ± 0.10

ND: Not Determined.

significant on the intrinsic pathway [47], Table 3. Once again, all of the compound examined exhibited a larger increase in active caspases 8 and 9 levels than the control doxorubicin.

2.2.3.2. Cytochrome C assay. Cytochrome *C* levels within the cell are critical for activating caspases and conducting the apoptotic process [47]. Table 3 shows the findings of testing indazole derivatives **6f, 6n,** and **6 s** as cytochrome *C* activators in the MCF-7 human breast cancer cell line. Indazoles **6f, 6n,** and **6 s** increased cytochrome *C* levels in the MCF-7 human breast cancer cell line by approximately 18, 16.5, and 17 times, respectively, compared to untreated control cells. The findings

add to the evidence that apoptosis can be attributed to cytochrome *C* overexpression and activation of the intrinsic apoptotic pathway generated by the investigated compounds.

2.2.3.3. Bax and Bcl-2 levels assay. The effects of the indazole derivatives **6f**, **6n**, and **6 s** on Bax and Bacl-2 levels in a breast cancer cell line (MCF-7) were investigated further using PCR approach and doxorubicin as a control [48]. Table 4 shows that the investigated compounds induced a considerable rise in Bax levels when compared to doxorubicin. Compound **6f** revealed a 35-fold increase in Bax level (297 pg/mL) compared to doxorubicin (276 pg/mL, 32-fold increase), followed by compound **6n** (281 pg/mL and 33-fold change) and at last **6 s** (279 pg/mL and 33-fold change). Finally, compound **6f** reduced Bcl-2 protein levels in MCF-7 cells to 0.840 ng/mL, followed by compounds **6n** (0.940 ng/mL) and **6 s** (0.960 ng/mL) compared to doxorubicin (0.985 ng/mL).

2.3. Molecular docking studies

As shown in the enzymatic assays run against CDK2, EGFR, and c-Met, that compounds **6f**, **6n**, and **6 s** were the most potent inhibitors for such enzymes, so we explored their binding interactions within crystal structure of previous targets. Molecular docking studies were run using MOE software within crystal structure of CDK2 (PDB ID: 1PYE), EGFR (PDB ID: 1 M17), and c-Met (PDB ID: 3DKF); respectively, and their binding interactions energy and root-mean square deviations were reported in Table 5 [49,50]. Interestingly, all tested compounds (**6f**, **6n**, **and 6 s)** showed higher binding energy over co-crystallized ligand within CDK2 active site, while on the contrary on EGFR and c-Met was seen, even their binding score energy still of high value to explain their inhibitory activity against such enzymes.

2D binding interactions of test compounds showed their efficient occupancy of active sites of 3 used crystal structures with number of binding interactions (varying from H- and pi-H bonding), as shown in supporting information file. Visual inspection of compound **6f** interaction diagram within 1PYE active site showed its perfect overlay with cocrystallized ligand (as shown in Fig. 4), in addition to a number of Hbonding and pi-H binding interactions (as listed in Table 5) with key amino acids lining 1PYE active site.

On the other hand, exploring the binding interactions within EGFR active site (PDB ID: 1 M17) of all test compounds **6f**, **6n**, and **6 s**, revealed the strong interactions exhibited by **6n** with key amino acids lining EGFR active site compared with that of co-crystallized ligand (as shown in Fig. 5).

Finally, examining the molecular docking simulations of compounds **6f**, **6n**, and **6 s** within c-Met crystal structure (PDB ID: 3DKF) revealed interesting observations: a common pi-pi interaction with TYR1230, in addition to a H-acceptor interaction with MET 1160 amino acid residue similar to these shown with co-crystallized ligand (as shown in Fig. 6). The best overlay with co-crystallized ligand was found with compounds **6n** and **6 s** (as shown in Fig. 6). In conclusion, molecular dynamic simulations of compounds 6f, 6n, and 6 s completely coincided with the observed actions against CDK2, EGFR, and c-Met target proteins, and this might be used to further investigate their mechanistic investigations as prospective anti-cancer therapy agents.

3. Conclusion

A series of new indazole derivatives **6a-v**, which are differently substituted at the 6-position of the indazole moiety have been developed. Twenty two target compounds were synthesized and tested *in vitro* against a panel of cancer cell lines and three different targets namely CDK-2, EGFR, and c-Met. The majority of the compounds examined had improved antiproliferative inhibitory activity. The most potent compounds were **6f**, **6i**, **6j**, **6 s**, and **6n**. Compounds **6j**, **6n**, and **6 s** demonstrated doxorubicin-like activity against the four cell lines and effectively inhibited these three targets. Compounds **6j**, **6n** and **6 s** also induced apoptosis. Molecular docking experiments were carried out to

Fig. 3. Caspase-3 level for compounds **6f**, **6n**, **6 s** and **doxorubicin** in human breast cancer cell line (MCF-7).

Table 3

Effects of compounds **6f**, **6n**, **6 s** and doxorubicin on active Caspases 3, 8, 9 and Cytochrome *C* in MCF-7 breast cancer cell line.

Comp. Code	Caspase-3		Caspase-8		Caspase-9		Cytochrome *C*	
	Conc (pg/ml)	Fold change	Conc (ng/ml)	Fold change	Conc (ng/ml)	Fold change	Conc (ng/ml)	Fold change

6f 602.40 ± 6.5 9 1.95 ± 0.10 11.5 18.8 ± 1.50 20 0.825 ± 0.08 18
6n 505.30 ± 4.0 7.70 1.80 ± 0.10 10.5 16.9 ± 1.50 18 0.765 ± 0.06 16.5
6 s 510.50 ± 4.5 7.80 1.87 ± 0.10 11 17.1 ± 1.50 18.5 0.795 ± 0.07 17.3
Doxorubicin 503.20 ± 4.2 7.65 1.75 ± 0.10 10.0 16.2 ± 1.50 17.4 0.604 ± 0.05 13.1
Control 65.60 1 0.17 1 0.93 1 0.046 1

Table 4

Bax and Bcl-2 levels for compounds **6f, 6n, 6 s** and **Doxorubicin** in MCF-7 breast cancer cell line.

Comp. Code	Bax		Bcl-2	
	Conc (pg/ml)	Fold change	Conc (ng/ml)	Fold reduction
6f	297.50 ± 30	35	0.840 ± 0.05	6
6n	281.40 ± 27	33	0.940 ± 0.07	5.5
6 s	278.90 ± 25	32.8	0.960 ± 0.07	5.3
Doxorubicin	276.50 ± 25	32.5	0.985 ± 0.07	5.1
Cont.	8.50	1	5.080	1.00

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Table 5

Binding Interactions of **6f**, **6n**, & **6 s** within CDK2, EGFR, & c-Met active site (PDB ID: 1PYE, 1 M17, & 3DKF; respectively).

	CDK2 (PDB ID: 1PYE)			EGFR (PDB ID: 1 M17)			c-Met (PDB ID: 3DKF)		
#	S_b	$RMSD_c$	Ligand Interactions	S	RMSD	Ligand Interactions	S	RMSD	Ligand Interactions
			a. a. Type Length			a. a. Type Length			a. a. Type Length
-a	□ 5.9	1.84	GLU H(d)d 3.05	□ 7.3	1.3	GLN 767 H(d) 3.15	□ 8.4	0.73	ARG 1208 H(d) 3.39
									ARG 1208 H(d) 3.41
									ASP 1222 H(a) 2.91
			LEU 83 H(a)e 3.07			MET 769 H(a) 2.7			MET 1160 H(a) 2.93
			TYR 1230 pi-pi 3.80						
6f	□ 6.43	2.04	LEU 83 H(a) 3.11	□ 6.34	1.15	MET 769 H(d) 3.47	□ 7.1	1.61	MET 1160 H(a) 3
			LEU 83 H(d) 3.33			TYR 1230 pi-pi 3.83			
			ALA 144 pi-H 4.03			TYR 1230 pi-pi 3.7			
6n	□ 6.2	1.73	LEU 83 H(d) 2.9	□ 6.44	1.96	MET 742 H(d) 3.43	□ 6.04	1.41	MET 1160 H(a) 3.04
			GLU 738 H(d) 3.35						
			GLN 131 H(d) 3.09			LYS 721 H(a) 3.46			TYR 1230 pi-pi 3.72
			LYS 721 pi-H 3.7						
6 s	□ 6.34	2.02	LEU 83 H(a) 3.28	□ 5.4	2.01	MET 769 H(a) 3.45	□ 6.75	1.9	ASN 1209 H(a) 3.68
			ILE 1084 pi-H 4.33						
			TYR 1230 pi-pi 3.95						
			TYR 1230 pi-pi 3.55						

a co-crystallized ligand; b docking score (Kcal/mol); c root-mean square deviation (Å); d H-donor; e H-acceptor.

Fig. 4. Schematic diagram of binding interactions of compound **6f** (purple) and co-crystallized ligand (yellow) within CDK2 active site (PDB ID: 1PYE).

Fig. 5. Schematic Diagram of binding Interactions of compound **6n** within EGFR active site (PDB ID: 1 M17) showing H-bonds (yellow-colored) and pi-H bonds (orange-colored).

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explore the novel compounds' binding mechanisms, affinities, and interactions with target receptors. The results demonstrated that the novel compounds fit nicely to the active sites of these targets, with high binding scores.

4. Experimental

4.1. Chemistry

4.1.1. General procedures for the synthesis of amidoximes 6a-v

To a solution of 1*H*-indazole-6-carboxylic acid **5** (1 mmol) in DMF (5 mL), EDC (1.3 mmol) and HOAt (1 mmol) were added. The amidoximes **3a-v** (1 mmol) were added after 30 min of stirring at room temperature. The resulting mixture was stirred for 24 h at room temperature. The mixture was extracted with EtOAc after adding 30 mL of water, washed three times with water, and once with brine. The mixed organic layer was dried with Na2SO4 and then evaporated at reduced pressure. Finally, using silica gel column chromatography, the resulting compounds **6a-v** were purified and isolated as white powders in 50–70% yields.

4.1.2. (Z)-N'-[(1H-indazole-6-carbonyl) oxy]benzo[d][1,3]dioxole-5-carboximidamide (6a)

Yield 55%, white solid, m.p. 223–225 °C. ^{1}H NMR (400 MHz, DMSO‑d6) δ 13.47 (s, 1H), 8.38 (s, 1H), 8.21 (s, 1H), 7.89 (s, 2H), 7.35 (dd, *J* = 8.1, 1.5 Hz, 1H), 7.31 (d, *J* = 1.4 Hz, 1H), 7.03 (d, *J* = 8.1 Hz, 1H), 6.92 (s, 2H), 6.11 (s, 2H). ^{13}C NMR (101 MHz, DMSO‑d6) δ 164.05, 156.59, 149.08, 147.24, 139.24, 133.70, 126.96, 125.39, 125.22, 121.25, 120.69, 120.51, 112.46, 108.10, 107.00, 101.53. HRMS (EI):

calcd. for $C_{16}H_{12}N_4NaO_4$ [M + Na]+: m/z 347.0756; found: m/z 347.0761.

4.1.3. (Z)-N'-[(1H-indazole-6-carbonyl)oxy]isonicotinimidamide (6b)
Yield 53%, white solid, m.p. 230–232 °C. 1H NMR (400 MHz, DMSO‑d_6) δ 13.50 (s, 1H), 8.73 (d, J = 5.9 Hz, 2H), 8.42 (s, 1H), 8.23 (s, 1H), 7.91 (s, 2H), 7.78 (d, J = 6.0 Hz, 2H), 7.26 (s, 2H). 13C NMR (101 MHz, DMSO‑d_6) δ 163.90, 155.14, 150.05, 139.23, 133.73, 126.57, 125.32, 121.12, 120.72, 120.61, 112.64. HRMS (EI): calcd. for $C_{14}H_{12}N_5O_2$ [M + H]+: m/z 282.0991; found: m/z 282.0996.

4.1.4. (Z)-N'-[(1H-indazole-6-carbonyl)oxy]-5-(hydroxymethyl)furan-3-carboximidamide (6c)
Yield 50 %, white solid, m.p. 139–142 °C. 1H NMR (400 MHz, DMSO‑d_6) δ 13.48 (s, 1H), 8.40 (s, 1H), 8.22 (s, 1H), 7.90 (s, 2H), 7.07 (d, J = 3.3 Hz, 1H), 6.94 (s, 2H), 6.46 (d, J = 3.2 Hz, 1H), 5.42 (t, J = 5.3 Hz, 1H), 4.47 (d, J = 4.6 Hz, 2H). 13C NMR (101 MHz, DMSO‑d_6) δ 163.86, 157.19, 149.57, 143.87, 139.27, 133.74, 126.69, 125.24, 120.70, 120.56, 112.57, 112.23, 108.49, 55.65. HRMS (EI): calcd. for $C_{14}H_{12}N_4NaO_4$ [M + Na]+: m/z 323.0756; found: m/z 323.0759.

4.1.5. (Z)-N'-[(1H-indazole-6-carbonyl)oxy]picolinimidamide (6d)
Yield 58%, beige solid, m.p. 175–176 °C. 1H NMR (400 MHz, DMSO‑d_6) δ 13.50 (s, 1H), 8.70 (d, J = 4.5 Hz, 1H), 8.46 (s, 1H), 8.23 (s, 1H), 8.06 (d, J = 7.9 Hz, 1H), 7.94 (dt, J = 11.4, 8.1 Hz, 1H), 7.70–7.50 (m, 1H), 7.18 (s, 1H). 13C NMR (101 MHz, DMSO‑d_6) δ 163.88, 154.41, 148.73, 148.27, 139.23, 137.33, 133.72, 126.62, 125.75, 125.31, 121.11, 120.74, 120.56, 112.66. HRMS (EI): calcd. for $C_{14}H_{11}N_5NaO_2$ [M + Na]+: m/z 304.0810; found: m/z 304.0809.

4.1.6. (Z)-N'-[(1H-indazole-6-carbonyl) oxy]thiophene-3-carboximidamide (6e)
Yield 60%, white solid, m.p. 198–202 °C. 1H NMR (400 MHz, DMSO‑d_6) δ 13.47 (s, 1H), 8.39 (s, 1H), 8.22 (s, 1H), 8.13 (d, J = 1.8 Hz, 1H), 7.93–7.83 (m, 1H), 7.65 (dd, J = 5.0, 2.9 Hz, 1H), 7.49 (dd, J = 5.0, 0.9 Hz, 1H), 6.96 (s, 1H). 13C NMR (101 MHz, DMSO‑d_6) δ 164.00, 153.26, 139.24, 133.71, 132.98, 126.92, 126.75, 126.09, 125.91, 125.23, 120.68, 120.55, 112.47. HRMS (EI): calcd. for $C_{13}H_{10}N_4NaO_2S$ [M + Na]+: m/z 309.0422; found: m/z 309.0418.

4.1.7. (Z)-N'-[(1H-indazole-6-carbonyl)oxy]-3,5-dimethoxybenzimidamide (6f)
Yield 56%, white solid, m.p. 223–225 °C. 1H NMR (400 MHz, DMSO‑d_6) δ 13.47 (s, 1H), 8.39 (s, 1H), 8.22 (s, 1H), 7.90 (s, 2H), 7.00 (s, 2H), 6.95 (d, J = 2.2 Hz, 2H), 6.66 (t, J = 2.1 Hz, 1H), 3.81 (s, 6H). 13C NMR (101 MHz, DMSO‑d_6) δ 164.06, 160.27, 156.90, 139.23, 133.71, 133.66, 126.91, 125.24, 120.70, 120.54, 112.49, 104.91, 102.40, 55.40. HRMS (EI): calcd. for $C_{17}H_{16}N_4NaO_4$ [M + Na]+: m/z 363.1069; found: m/z 363.1072.

4.1.8. (Z)-N'-[(1H-indazole-6-carbonyl)oxy]-4-nitrobenzimidamide (6 g)
Yield 50%, white solid, m.p. 204–206 °C. 1H NMR (400 MHz, DMSO‑d_6) δ 13.50 (s, 1H), 8.43 (s, 1H), 8.36 (d, J = 8.8 Hz, 2H), 8.23 (s, 1H), 8.08 (d, J = 8.8 Hz, 2H), 7.91 (s, 2H), 7.31 (s, 2H). 13C NMR (101 MHz, DMSO‑d_6) δ 163.91, 155.45, 148.66, 139.23, 137.89, 133.74, 128.35, 126.57, 125.33, 123.56, 120.73, 120.61, 112.65. HRMS (EI): calcd. for $C_{15}H_{11}N_5NaO_4$ [M + Na]+: m/z 348.0709; found: m/z 348.0712.

Fig. 6. Schematic diagram of binding interactions of compound **6n** (yellow), **6 s** (green) and co-crystallized ligand (purple) within c-Met active site (PDB ID: 3DKF).

4.1.9. (Z)-N'-[(1H-indazole-6-carbonyl)oxy]-3,4,5-trimethoxybenzimidamide (6 h)
Yield 52%, white solid, m.p. 210–213 °C. 1H NMR (400 MHz, DMSO‑d_6) δ 13.47 (s, 1H), 8.38 (s, 1H), 8.22 (s, 1H), 7.89 (q, J = 8.6 Hz, 2H), 7.09 (s, 2H), 6.98 (s, 2H), 3.85 (s, 6H), 3.72 (s, 3H). 13C NMR (101 MHz, DMSO‑d_6) δ 164.10, 156.96, 152.65, 147.81, 139.31, 139.24, 133.70, 127.00, 125.23, 120.67, 120.55, 112.44, 104.40, 60.09, 56.01. HRMS (EI): calcd. for $C_{18}H_{18}N_4NaO_5$ [M + Na]+: m/z 393.1175; found: m/z 393.1179.

4.1.10. (Z)-N'-[(1H-indazole-6-carbonyl)oxy]-2-naphthimidamide (6i)
Yield 54%, white solid, m.p. 210–212 °C. 1H NMR (400 MHz, DMSO‑d_6) δ 13.49 (s, 1H), 8.43 (d, J = 11.2 Hz, 1H), 8.23 (s, 1H), 8.02

(dt, *J* = 9.6, 5.8 Hz, 2H), 7.92 (dd, *J* = 7.6, 3.1 Hz, 2H), 7.67–7.56 (m, 1H), 7.16 (s, 1H). 13C NMR (101 MHz, DMSO‑*d6*) δ 164.08, 156.90, 133.80, 132.30, 129.12, 128.48, 127.87, 127.61, 127.24, 126.91, 126.69, 126.65, 125.26, 124.13, 120.74, 120.57, 112.55. HRMS (EI): calcd. for $C_{19}H_{14}N_4NaO_2$ [M + Na]+: *m/z* 353.1014; found: *m/z* 353.1014.

4.1.11. (Z)-N'-[(1H-indazole-6-carbonyl)oxy]-4-chlorobenzimidamide (6j)

Yield 56%, white solid, m.p. 220–222 ◦C. 1H NMR (400 MHz, DMSO‑*d6*) δ 13.51 (s, 1H), 8.41 (s, 1H), 8.22 (s, 1H), 7.90 (s, 2H), 7.83 (d, *J* = 8.5 Hz, 2H), 7.57 (d, *J* = 8.5 Hz, 2H), 7.10 (s, 2H). 13C NMR (101 MHz, DMSO‑*d6*) δ 163.99, 156.06, 139.28, 135.21, 133.66, 130.58, 128.73, 128.46, 126.79, 125.24, 120.70, 120.54, 112.62. HRMS (EI): calcd. for $C_{15}H_{11}ClN_4NaO_2$ [M + Na]+: *m/z* 337.0468; found: *m/z* 337.0470.

4.1.12. (Z)-N'-[(1H-indazole-6-carbonyl)oxy]-4-bromobenzimidamide (6 k)

Yield 60%, white solid, m.p. 252–255 ◦C. 1H NMR (400 MHz, DMSO‑*d6*) δ 13.48 (s, 1H), 8.40 (s, 1H), 8.22 (s, 1H), 7.90 (s, 1H), 7.73 (dd, *J* = 19.3, 8.6 Hz, 2H), 7.10 (s, 1H). 13C NMR (101 MHz, DMSO‑*d6*) δ 163.99, 156.16, 139.23, 133.71, 131.40, 130.95, 128.96, 126.79, 125.27, 123.97, 120.71, 120.56, 112.54. HRMS (EI): calcd. for $C_{15}H_{11}BrN_4NaO_2$ [M + Na]+: *m/z* 380.9963; found: *m/z* 380.9967.

4.1.13. (Z)-N'-[(1H-indazole-6-carbonyl)oxy]-4-fluorobenzimidamide (6 l)

Yield 50%, white solid, m.p. 206–209 ◦C. 1H NMR (400 MHz, DMSO‑*d6*) δ 13.48 (s, 1H), 8.40 (s, 1H), 8.22 (s, 1H), 7.90 (s, 1H), 7.86 (dd, *J* = 8.7, 5.5 Hz, 1H), 7.34 (t, *J* = 8.9 Hz, 1H), 7.06 (s, 1H). 13C NMR (101 MHz, DMSO‑*d6*) δ 164.04, 156.21, 139.24, 133.71, 129.34, 129.26, 128.15, 126.86, 125.25, 120.70, 120.55, 115.46, 115.24. HRMS (EI): calcd. for $C_{15}H_{11}FN_4NaO_2$ [M + Na]+: *m/z* 321.0764; found: *m/z* 321.0765.

4.1.14. (Z)-N'-[(1H-indazole-6-carbonyl)oxy]-4-iodobenzimidamide (6 m)

Yield 58%, white solid, m.p. 254–255 ◦C. 1H NMR (400 MHz, DMSO‑*d6*) δ 13.48 (s, 1H), 8.40 (s, 1H), 8.22 (s, 1H), 7.94–7.77 (m, 2H), 7.59 (d, *J* = 8.4 Hz, 1H), 7.07 (s, 1H). 13C NMR (101 MHz, DMSO‑*d6*) δ 163.98, 156.35, 139.23, 137.23, 133.71, 131.26, 128.85, 126.80, 125.26, 120.70, 120.55, 112.53, 97.50. HRMS (EI): calcd. for $C_{15}H_{11}IN_4NaO_2$ [M + Na]+: *m/z* 428.9824; found: *m/z* 428.9827.

4.1.15. (Z)-N'-[(1H-indazole-6-carbonyl)oxy]-6-methoxynicotinimidamide (6n)

Yield 50%, white solid, m.p. 219–223 ◦C. 1H NMR (400 MHz, DMSO‑*d6*) δ 13.48 (s, 1H), 8.59 (d, *J* = 1.7 Hz, 1H), 8.39 (s, 1H), 8.22 (s, 1H), 8.07 (dd, *J* = 8.6, 2.0 Hz, 1H), 7.90 (s, 2H), 7.08 (s, 2H), 6.94 (d, *J* = 8.7 Hz, 1H), 3.92 (s, 3H). 13C NMR (101 MHz, DMSO‑*d6*) δ 164.87, 164.03, 155.11, 145.67, 139.23, 137.73, 133.71, 126.85, 125.26, 121.20, 120.69, 120.56, 112.49, 110.25, 53.55. HRMS (EI): calcd. for $C_{15}H_{13}N_5NaO_3$ [M + Na]+: *m/z* 334.0916; found: *m/z* 334.0913.

4.1.16. (Z)-N'-[(1H-indazole-6-carbonyl)oxy]-1H-pyrazole-3-carboximidamide (6o)

Yield 50%, white solid, m.p. 230–233 ◦C. 1H NMR (400 MHz, DMSO‑*d6*) δ 13.37 (d, *J* = 79.2 Hz, 1H), 8.52–8.05 (m, 1H), 7.99–7.71 (m, 1H), 6.71 (d, *J* = 64.5 Hz, 1H). 13C NMR (101 MHz, DMSO‑*d6*) δ 164.00, 153.26, 139.24, 133.71, 132.98, 126.92, 126.75, 126.09, 125.91, 125.23, 120.68, 120.55, 112.47. HRMS (EI): calcd. for $C_{12}H_{10}N_6NaO_2$ [M + Na]+: *m/z* 293.0763; found: *m/z* 293.0759.

4.1.17. (Z)-N'-[(1H-indazole-6-carbonyl)oxy]-1-oxo-1,3-dihydroisobenzofuran-5-carboximidamide (6p)

Yield 55%, white solid, m.p. 243–245 ◦C. 1H NMR (400 MHz, DMSO‑*d6*) δ 13.49 (s, 1H), 8.43 (s, 1H), 8.23 (s, 1H), 8.09 (s, 1H), 7.99 (d, *J* = 3.2 Hz, 2H), 7.91 (s, 2H), 7.26 (s, 2H), 5.50 (s, 2H). 13C NMR (101 MHz, DMSO‑*d6*) δ 170.08, 163.93, 156.28, 147.43, 139.23, 137.28, 133.73, 127.80, 126.64, 125.32, 124.98, 121.59, 120.73, 120.59, 112.62, 69.96. HRMS (EI): calcd. for $C_{17}H_{12}N_4NaO_4$ [M + Na]+: *m/z* 359.0756; found: *m/z* 359.3175.

4.1.18. (Z)-N'-[(1H-indazole-6-carbonyl)oxy]benzimidamide (6q)

Yield 58%, white solid, m.p. 196–198 ◦C. 1H NMR (400 MHz,

DMSO‑d_6) δ 13.49 (s, 1H), 8.43 (d, *J* = 10.5 Hz, 1H), 8.23 (s, 1H), 8.02 (dt, *J* = 9.5, 5.7 Hz, 2H), 7.92 (dd, *J* = 6.4, 1.6 Hz, 1H), 7.67–7.56 (m, 1H), 7.16 (s, 1H). ^{13}C NMR (101 MHz, DMSO‑d_6) δ 163.99, 156.16, 139.23, 133.71, 131.40, 130.95, 128.96, 126.79, 125.27, 123.97, 120.71, 120.56, 112.54. HRMS (EI): calcd. for $C_{15}H_{13}N_4O_2$ [M + H]+: *m/z* 281.1039; found: *m/z* 281.0835.

4.1.19. (Z)-N'-[(1H-indazole-6-carbonyl)oxy]-4-methylbenzimidamide (6r)

Yield 60%, white solid, m.p. 186–189 ∘C. ^{1}H NMR (400 MHz, DMSO‑d_6) δ 13.47 (s, 1H), 8.40 (s, 1H), 8.22 (s, 1H), 7.89 (s, 2H), 7.70 (d, *J* = 8.1 Hz, 2H), 7.30 (d, *J* = 8.1 Hz, 2H), 6.95 (s, 2H), 2.37 (s, 3H). ^{13}C NMR (101 MHz, DMSO‑d_6) δ 164.08, 156.94, 140.22, 139.25, 133.70, 128.89, 128.85, 126.99, 126.76, 125.21, 120.70, 120.52, 112.47, 20.89. HRMS (EI): calcd. for $C_{16}H_{14}N_4NaO_2$ [M + Na]+: *m/z* 317.1014; found: *m/z* 317.1012.

4.1.20. (Z)-N-(4-(N'-[(1H-indazole-6-carbonyl)oxy]carbamimidoyl) phenyl)acetamide (6 s)

Yield 54%, white solid, m.p. 194–197 ∘C. ^{1}H NMR (400 MHz, DMSO‑d_6) δ 13.47 (s, 1H), 10.16 (s, 1H), 8.39 (s, 1H), 8.22 (s, 1H), 7.89 (s, 2H), 7.74 (d, *J* = 8.7 Hz, 2H), 7.68 (d, *J* = 8.7 Hz, 2H), 6.93 (s, 2H), 2.08 (s, 3H). ^{13}C NMR (101 MHz, DMSO‑d_6) δ 168.62, 164.09, 156.66, 141.28, 133.68, 127.45, 127.00, 125.95, 125.19, 124.50, 120.69, 120.53, 118.34, 112.50, 24.05. HRMS (EI): calcd. for $C_{17}H_{15}N_5NaO_3$ [M + Na]+: *m/z* 360.1073; found: *m/z* 360.1073.

4.1.21. (Z)-N'-[(1H-indazole-6-carbonyl)oxy]furan-2-carboximidamide (6 t)

Yield 50%, white solid, m.p. 187–189 ∘C. ^{1}H NMR (400 MHz, DMSO‑d_6) δ 13.48 (s, 1H), 8.40 (s, 1H), 8.22 (s, 1H), 7.88 (d, *J* = 10.1 Hz, 3H), 7.10 (d, *J* = 3.4 Hz, 1H), 7.00 (s, 2H), 6.66 (dd, *J* = 3.3, 1.7 Hz, 1H). ^{13}C NMR (101 MHz, DMSO‑d_6) δ 163.87, 149.64, 144.82, 144.61, 139.26, 133.75, 126.68, 125.25, 120.70, 120.57, 112.57, 111.56, 111.37. HRMS (EI): calcd. for $C_{13}H_{10}N_4NaO_3$ [M + Na]+: *m/z* 293.0651; found: *m/z* 293.0651.

4.1.22. (Z)-N'-[(1H-indazole-6-carbonyl)oxy]cinnamimidamide (6u)

Yield 52%, white solid, m.p. 198–201 ∘C. ^{1}H NMR (400 MHz, DMSO‑d_6) δ 13.48 (s, 1H), 8.40 (s, 1H), 8.22 (s, 1H), 7.89 (s, 2H), 7.61 (d, *J* = 7.3 Hz, 2H), 7.50–7.33 (m, 4H), 6.81 (s, 1H), 6.60 (d, *J* = 16.7 Hz, 1H). ^{13}C NMR (101 MHz, DMSO‑d_6) δ 163.82, 155.83, 139.24, 135.40, 134.70, 133.71, 128.97, 128.91, 127.06, 126.78, 125.27, 120.69,

120.57, 119.33, 112.53. HRMS (EI): calcd. for $C_{17}H_{14}N_4NaO_2$ [M + Na]+: *m/z* 329.1014; found: *m/z* 329.1016.

4.1.23. (Z)-N'-[(1H-indazole-6-carbonyl)oxy]-1-naphthimidamide (6v)

Yield 50%, white solid, m.p. 152–156 ∘C. ^{1}H NMR (400 MHz, DMSO‑d_6) δ 13.51 (s, 1H), 8.45 (s, 1H), 8.36 (d, *J* = 8.1 Hz, 1H), 8.23 (s, 1H), 8.08 (d, *J* = 8.1 Hz, 1H), 8.02 (d, *J* = 7.4 Hz, 1H), 7.93 (s, 2H), 7.69 (d, *J* = 6.5 Hz, 2H), 7.62 (dd, *J* = 14.2, 6.4 Hz, 4H), 7.23 (s, 2H). ^{13}C NMR (101 MHz, DMSO‑d_6) δ 164.26, 157.84, 133.71, 133.05, 130.83, 130.22, 129.84, 128.11, 127.06, 126.74, 126.25, 125.46, 125.22, 125.13, 120.76, 120.57, 112.58. HRMS (EI): calcd. for $C_{19}H_{14}N_4NaO_2$ [M + Na]+: *m/z* 353.1014; found: *m/z* 353.1015.

4.2. Biology

All experimental techniques for the MTT assay, *in vitro* enzymatic assays of CDK-2, EGFR, and c-Met, and Apoptosis are detailed in Appendix A.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2022.105922.

References

[1] G.F. Zha, H.L. Qin, B.G.M. Youssif, M.W. Amjad, M.A.G. Raja, A.H. Abdelazeem, S. N.A. Bukhari, Discovery of potential anticancer multi-targeted ligustrazine based cyclohexanone and oxime analogs overcoming the cancer multidrug resistance, Eur. J. Med. Chem. 135 (2017) 34–48, https://doi.org/10.1016/j.ejmech.2017.04.025.

[2] M. Hisham, B.G.M. Youssif, E.E.A. Osman, A.M. Hayallah, M. Abdel-Aziz, Synthesis and biological evaluation of novel xanthine derivatives as potential apoptotic antitumor agents, Eur. J. Med. Chem. 176 (2019) 117–128, https://doi.org/10.1016/j.ejmech.2019.05.015.

[3] D. Hughes, D.I. Andersson, Evolutionary consequences of drug resistance: Shared principles across diverse targets and organisms, Nat. Rev. Genet. 16 (8) (2015) 459–471, https://doi.org/10.1038/nrg3922.

[4] J. Leh´ar, A.S. Krueger, W. Avery, A.M. Heilbut, L.M. Johansen, E.R. Price, R. J. Rickles, G.F. Short III, J.E. Staunton, X. Jin, M.S. Lee, G.R. Zimmermann, A. A. Borisy, Synergistic drug combinations tend to improve therapeutically relevant selectivity, Nat. Biotechnol. 27 (7) (2009) 659–666, https://doi.org/10.1038/nbt.1549.

[5] K.N. Shah, R. Bhatt, J. Rotow, J. Rohrberg, V. Olivas, V.E. Wang, G. Hemmati, M. M. Martins, A. Maynard, J. Kuhn, J. Galeas, H.J. Donnella, S. Kaushik, A. Ku, S. Dumont, G. Krings, H.J. Haringsma, L. Robillard, A.D. Simmons, T.C. Harding, F. McCormick, A. Goga, C.M. Blakely, T.G. Bivona, S. Bandyopadhyay, Aurora kinase A drives the evolution of resistance to third-generation EGFR inhibitors in lung cancer, Nat. Med. 25 (1) (2019) 111–118, https://doi.org/10.1038/s41591-018-0264-7.

[6] D.D. Gaikwad, A.D. Chapolikar, C.G. Devkate, K.D. Warad, A.P. Tayade, R. P. Pawar, A.J. Domb, Synthesis of indazole motifs and their medicinal importance: An overview, Eur. J. Med. Chem. 90 (2015) 707–731, https://doi.org/10.1016/j.ejmech.2014.11.029.

[7] I. Denya, S.F. Malan, J. Joubert, Indazole derivatives and their therapeutic applications: a patent review (2013–2017), Expert Opin. Ther. Pat. 28 (6) (2018) 441–453, https://doi.org/10.1080/13543776.2018.1472240.

[8] S.-G. Zhang, C.-G. Liang, W.-H. Zhang, Recent advances in indazole-containing derivatives: Synthesis and biological perspectives, Molecules 23 (11) (2018) 2783, https://doi.org/10.3390/molecules23112783.

[9] J. Dong, Q. Zhang, Z. Wang, G. Huang, S. Li, Recent Advances in the Development of Indazole-based Anticancer Agents, ChemMedChem 13 (15) (2018) 1490–1507, https://doi.org/10.1002/cmdc.201800253.

[10] S.J. Yan, Y. Dong, Q. Peng, Q.Y. Fan, J.H. Zhang, J. Lin, Synthesis of polyhalo 2-aryl-4-aminoquinazolines and 3-amino-indazoles as anti-cancer agents, RSC Adv. 3 (2013) 5563–5569, https://doi.org/10.1039/c3ra23059g.

[11] G. Sandeep Reddy, S. Mohanty, J. Kumar, B. Venteswar Rao, Synthesis and Evaluation of Anticancer Activity of Indazole Derivatives, Russ. J. Gen. Chem. 88 (11) (2018) 2394–2399, https://doi.org/10.1134/S1070363218110233.

[12] P.A. Harris, A. Boloor, M. Cheung, R. Kumar, R.M. Crosby, R.G. Davis-Ward, A. H. Epperly, K.W. Hinkle, R.N. Hunter, J.H. Johnson, V.B. Knick, C.P. Laudeman, D. K. Luttrell, R.A. Mook, R.T. Nolte, S.K. Rudolph, J.R. Szewczyk, A.T. Truesdale, J. M. Veal, L. Wang, J.A. Stafford, Discovery of 5-[[4-[(2,3-dimethyl-2H-indazol-6-yl) methylamino]-2- pyrimidinyl]amino]-2-methyl-benzenesulfonamide (pazopanib), a novel and potent vascular endothelial growth factor receptor inhibitor, J. Med. Chem. 51 (15) (2008) 4632–4640, https://doi.org/10.1021/jm800566m.

[13] S. Miyamoto, S. Kakutani, Y. Sato, A. Hanashi, Y. Kinoshita, A. Ishikawa, Drug review: Pazopanib, Jpn. J. Clin. Oncol. 48 (2018) 503–513, https://doi.org/10.1093/jjco/hyy053.

[14] B.W. Konicek, A.R. Capen, K.M. Credille, P.J. Ebert, B.L. Falcon, G.L. Heady, B.K. R. Patel, V.L. Peek, J.R. Stephens, J.A. Stewart, S.L. Stout, D.E. Timm, S.L. Um, M. D. Willard, I.H. Wulur, Y.i. Zeng, Y. Wang, R.A. Walgren, S.-C. Betty Yan, Merestinib (LY2801653) inhibits neurotrophic receptor kinase (NTRK) and suppresses growth of NTRK fusion bearing tumors, Oncotarget 9 (17) (2018) 13796–13806.

[15] S.B. Yan, S.L. Um, V.L. Peek, J.R. Stephens, W. Zeng, B.W. Konicek, L. Liu, J. R. Manro, V. Wacheck, R.A. Walgren, MET-targeting antibody (emibetuzumab) and kinase inhibitor (merestinib) as single agent or in combination in a cancer model bearing MET exon 14 skipping, Invest. New Drugs 36 (2018) 536–544, https://doi.org/10.1007/s10637-017-0545-x.

[16] A.R. He, R.B. Cohen, C.S. Denlinger, A. Sama, A. Birnbaum, J. Hwang, T. Sato, N. Lewis, M. Mynderse, M. Niland, J. Giles, J. Wallin, B. Moser, W. Zhang, R. Walgren, E.R. Plimack, First-in-Human Phase I Study of Merestinib, an Oral Multikinase Inhibitor, Patients with Advanced Cancer, Oncologist 24 (2019) e930–e942, https://doi.org/10.1634/theoncologist.2018-0411.

[17] Z.T. Al-Salama, S.J. Keam, Entrectinib: First Global Approval, Drugs. 79 (2019) 1477–1483, https://doi.org/10.1007/s40265-019-01177-y.

[18] Y. Umeyama, Y. Shibasaki, H. Akaza, Axitinib in metastatic renal cell carcinoma: Beyond the second-line setting, Future Oncol. 13 (2017) 1839–1852, https://doi.org/10.2217/fon-2017-0104.

[19] A. Bellesoeur, E. Carton, J. Alexandre, F. Goldwasser, O. Huillard, Axitinib in the treatment of renal cell carcinoma: Design, development, and place in therapy, Drug Des. Developm. Therapy. 11 (2017) 2801–2811, https://doi.org/10.2147/DDDT.S109640.

[20] G. Zhao, W.Y. Li, D. Chen, J.R. Henry, H.Y. Li, Z. Chen, M. Zia-Ebrahimi, L. Bloem, Y. Zhai, K. Huss, S. bin Peng, D.J. McCann, A novel, selective inhibitor of fibroblast growth factor receptors that shows a potent broad spectrum of antitumor activity in several tumor xenograft models, Molecular Cancer Therapeutics. 10 (2011) 2200–2210. https://doi.org/10.1158/1535-7163.MCT-11-0306.

[21] M. Michael, Y.J. Bang, Y.S. Park, Y.K. Kang, T.M. Kim, O. Hamid, D. Thornton, S. C. Tate, E. Raddad, J. Tie, A Phase 1 Study of LY2874455, an Oral Selective pan-FGFR Inhibitor, Patients with Advanced Cancer, Targeted Oncology. 12 (2017) 463–474, https://doi.org/10.1007/s11523-017-0502-9.

[22] J.M. Mason, D.C.C. Lin, X. Wei, Y. Che, Y. Yao, R. Kiarash, D.W. Cescon, G. C. Fletcher, D.E. Awrey, M.R. Bray, G. Pan, T.W. Mak, Functional characterization of CFI-400945, a polo-like kinase 4 inhibitor, as a potential anticancer agent, Cancer Cell 26 (2014) 163–176, https://doi.org/10.1016/j.ccr.2014.05.006.

[23] P.B. Sampson, Y. Liu, B. Forrest, G. Cumming, S.W. Li, N.K. Patel, L. Edwards, R. Laufer, M. Feher, F. Ban, D.E. Awrey, G. Mao, O. Plotnikova, R. Hodgson, I. Beletskaya, J.M. Mason, X. Luo, V. Nadeem, X. Wei, R. Kiarash, B. Madeira, P. Huang, T.W. Mak, G. Pan, H.W. Pauls, The discovery of polo-like kinase 4 inhibitors: Identification of (1 R,2 S)-2-((E)-4-(((cis)-2,6-Dimethylmorpholino) methyl)styryl)-1 H -indazol-6-yl)-5′-methoxyspiro[cyclopropane-1,3′-indolin]-2′-one (CFI-400945) as a potent, orally active antitumor agent, J. Med. Chem. 58 (2015) 147–169, https://doi.org/10.1021/jm5005336.

[24] Y. Zhao, X. Wang, PLK4: a promising target for cancer therapy, Journal of Cancer Research and Clinical, Oncology. 145 (2019) 2413–2422, https://doi.org/10.1007/s00432-019-02994-0.

[25] X. Zhang, C. Wei, H. Liang, L. Han, Polo-Like Kinase 4's Critical Role in Cancer Development and Strategies for Plk4-Targeted Therapy, Front. Oncol. 11 (2021), https://doi.org/10.3389/fonc.2021.587554.

[26] G. Recondo, M. Bahcall, L.F. Spurr, J. Che, B. Ricciuti, G.C. Leonardi, Y.C. Lo, Y. Y. Li, G. Lamberti, T. Nguyen, M.S.D. Milan, D. Venkatraman, R. Umeton, C. P. Paweletz, A. Albayrak, A.D. Cherniack, K.S. Price, S.R. Fairclough, M. Nishino, L. M. Sholl, G.R. Oxnard, P.A. Janne, M.M. Awad, Molecular Mechanisms of Acquired Resistance to MET Tyrosine Kinase Inhibitors in Patients with MET Exon 14–Mutant NSCLC, Clin. Cancer Res. 26 (2020) 2615–2625, https://doi.org/10.1158/1078-0432.CCR-19-3608.

[27] J. Engel, A. Richters, M. Getlik, S. Tomassi, M. Keul, M. Termathe, J. Lategahn, C. Becker, S. Mayer-Wrangowski, C. Grütter, N. Uhlenbrock, J. Krüll, N. Schaumann, S. Eppmann, P. Kibies, F. Hoffgaard, J. Heil, S. Menninger, S. Ortiz-Cuaran, J.M. Heuckmann, V. Tinnefeld, R.P. Zahedi, M.L. Sos, C. Schultz-Fademrecht, R.K. Thomas, S.M. Kast, D. Rauh, Targeting Drug Resistance in EGFR with Covalent Inhibitors: A Structure-Based Design Approach, J. Med. Chem. 58 (2015) 6844–6863, https://doi.org/10.1021/acs.jmedchem.5b01082.

[28] B.G.M. Youssif, M.H. Abdelrahman, A.H. Abdelazeem, M.A. abdelgawad, H.M. Ibrahim, O.I.A. Salem, M.F.A. Mohamed, L. Treambleau, S.N.A. Bukhari, Design, synthesis, mechanistic and histopathological studies of small-molecules of novel indole-2-carboxamides and pyrazino[1,2-a]indol-1(2H)-ones as potential anticancer agents effecting the reactive oxygen species production, Eur. J. Med. Chem. 146 (2018) 260–273. https://doi.org/10.1016/j.ejmech.2018.01.042.

[29] H.A.M. Gomaa, M.E. Shaker, S.I. Alzarea, O.M. Hendawy, F.A.M. Mohamed, A. M. Gouda, A.T. Ali, M.M. Morcoss, M.H. Abdelrahman, L. Trembleau, B.G. M. Youssif, Optimization and SAR investigation of novel 2,3-dihydropyrazino[1,2-a]indole-1,4-dione derivatives as EGFR and BRAFV600E dual inhibitors with

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potent antiproliferative and antioxidant activities, Bioorg. Chem. 120 (2022), 105616, https://doi.org/10.1016/j.bioorg.2022.105616.

[30] B.G.M. Youssif, A.M. Gouda, A.H. Moustafa, A.A. Abdelhamid, H.A.M. Gomaa, I. Kamal, A.A. Marzouk, Design and synthesis of new triarylimidazole derivatives as dual inhibitors of BRAFV600E/p38α with potential antiproliferative activity, J. Mol. Struct. 1253 (2022), 132218, https://doi.org/10.1016/j.molstruc.2021.132218.

[31] S.C. Karad, V.B. Purohit, R.P. Thummar, B.K. Vaghasiya, R.D. Kamani, P. Thakor, V.R. Thakkar, S.S. Thakkar, A. Ray, D.K. Raval, Synthesis and biological screening of novel 2-morpholinoquinoline nucleus clubbed with 1,2,4-oxadiazole motifs, Eur. J. Med. Chem. 126 (2017) 894–909, https://doi.org/10.1016/j.ejmech.2016.12.016.

[32] X. Ji, J. Guo, Y. Liu, A. Lu, Z. Wang, Y. Li, S. Yang, Q. Wang, Marine-Natural-Product Development: First Discovery of Nortopsentin Alkaloids as Novel Antiviral, Anti-phytopathogenic-Fungus, and Insecticidal Agents, Journal of Agricultural and Food, Chemistry. 66 (2018) 4062–4072, https://doi.org/10.1021/acs.jafc.8b00507.

[33] B. Ruan, Y. Zhang, S. Tadesse, S. Preston, A.C. Taki, A. Jabbar, A. Hofmann, Y. Jiao, J. Garcia-Bustos, J. Harjani, T.G. Le, S. Varghese, S. Teguh, Y. Xie, J. Odiba, M. Hu, R.B. Gasser, J. Baell, Synthesis and structure-activity relationship study of pyrrolidine-oxadiazoles as anthelmintics against Haemonchus contortus, Eur. J. Med. Chem. 190 (2020), https://doi.org/10.1016/j.ejmech.2020.112100.

[34] L.H. Al-Wahaibi, A.M. Gouda, O.F. Abou-Ghadir, O.I.A. Salem, A.T. Ali, H. S. Farghaly, M.H. Abdelrahman, L. Trembleau, H.H.M. Abdu-Allah, B.G.M. Youssif, Design and synthesis of novel 2,3-dihydropyrazino[1,2-a]indole-1,4-dione derivatives as antiproliferative EGFR and BRAFV600E dual inhibitors, Bioorg. Chem. 104 (2020), https://doi.org/10.1016/j.bioorg.2020.104260.

[35] R.A. Mekheimer, S.M.R. Allam, M.A. Al-Sheikh, M.S. Moustafa, S.M. Al-Mousawi, Y.A. Mostafa, B.H.G.M. Youssif, K.U. Sadek, Discovery of new pyrimido[5,4-c] quinolines as potential antiproliferative agents with multitarget actions: Rapid synthesis, docking, and ADME studies, Bioorg. Chem. 121 (2022), https://doi.org/10.1016/j.bioorg.2022.105693.

[36] M.H. Abdelrahman, A.S. Aboraia, B.G.M. Youssif, B.E.M. Elsadek, Design, synthesis and pharmacophoric model building of new 3-alkoxymethyl/3-phenyl indole-2-carboxamides with potential antiproliferative activity, Chem. Biol. Drug Des. 90 (2017) 64–82, https://doi.org/10.1111/cbdd.12928.

[37] M.M. Al-Sanea, L. Gotina, M.F.A. Mohamed, D.G.T. Parambi, H.A.M. Gomaa, B. Mathew, B.G.M. Youssif, K.S. Alharbi, Z.M. Elsayed, M.A. Abdelgawad, W. M. Eldehna, Design, synthesis and biological evaluation of new HDAC1 and HDAC2 inhibitors endowed with ligustrazine as a novel cap moiety, Drug Design, Development and Therapy. 14 (2020) 497–508, https://doi.org/10.2147/DDDT.S237957.

[38] S. Tadesse, E.C. Caldon, W. Tilley, S. Wang, Cyclin-Dependent Kinase 2 Inhibitors in Cancer Therapy: An Update, J. Med. Chem. 62 (2019) 4233–4251, https://doi.org/10.1021/acs.jmedchem.8b01469.

[39] S.J. Park, E. Kim, M. Yoo, J.Y. Lee, C.H. Park, J.Y. Hwang, J. du Ha, Synthesis and biological evaluation of N9-cis-cyclobutylpurine derivatives for use as cyclindependent kinase (CDK) inhibitors, Bioorg. Med. Chem. Lett. 27 (2017) 4399–4404, https://doi.org/10.1016/j.bmcl.2017.08.018.

[40] H.A. Yu, S.K. Tian, A.E. Drilon, L. Borsu, G.J. Riely, M.E. Arcila, M. Ladanyi, Acquired resistance of EGFR-mutant lung cancer to AT790M-specificEGFR inhibitor: Emergence of a third mutation (C797S) in the EGFR tyrosine kinase domain, JAMA Oncology. 1 (2015) 982–984, https://doi.org/10.1001/jamaoncol.2015.1066.

[41] E.J. Hanan, M. Baumgardner, M.C. Bryan, Y. Chen, C. Eigenbrot, P. Fan, X.H. Gu, H. La, S. Malek, H.E. Purkey, G. Schaefer, S. Schmidt, S. Sideris, I. Yen, C. Yu, T. P. Heffron, 4-Aminoindazolyl-dihydrofuro[3,4-d]pyrimidines as non-covalent inhibitors of mutant epidermal growth factor receptor tyrosine kinase, Bioorg. Med. Chem. Lett. 26 (2016) 534–539, https://doi.org/10.1016/j.bmcl.2015.11.078.

[42] A.M. Mohassab, H.A. Hassan, D. Abdelhamid, A.M. Gouda, B.G.M. Youssif, H. Tateishi, M. Fujita, M. Otsuka, M. Abdel-Aziz, Design and synthesis of novel quinoline/chalcone/1,2,4-triazole hybrids as potent antiproliferative agent targeting EGFR and BRAFV600E kinases, Bioorg. Chem. 106 (2021), https://doi.org/10.1016/j.bioorg.2020.104510.

[43] B. Qi, Y. Yang, H. He, X. Yue, Y. Zhou, X. Zhou, Y. Chen, M. Liu, A. Zhang, F. Wei, Identification of novel N1-(2-aryl-1, 3-thiazolidin-4-one)-N3-aryl ureas showing potent multi-tyrosine kinase inhibitory activities, Eur. J. Med. Chem. 146 (2018) 368–380, https://doi.org/10.1016/j.ejmech.2018.01.061.

[44] J.-Y. Yoon, J.-J. Lee, S. Gu, M.E. Jung, H.-S. Cho, J.H. Lim, S.Y. Jun, J.-H. Ahn, J.-S. Min, M.-H. Choi, S.-J. Jeon, Y.-J. Lee, A. Go, Y.-J. Heo, C.-R. Jung, G. Choi, K. Lee, M.-K. Jeon, N.-S. Kim, Oncotarget 1 www.impactjournals.com/oncotarget Novel indazole-based small compounds enhance TRAIL-induced apoptosis by inhibiting the MKK7-TIPRL interaction in hepatocellular carcinoma, n.d. www.impactjournals.com/oncotarget/.

[45] T. Nguyen, J.X. Li, B.F. Thomas, J.L. Wiley, T.P. Kenakin, Y. Zhang, Allosteric Modulation: An Alternate Approach Targeting the Cannabinoid CB1 Receptor, Med. Res. Rev. 37 (2017) 441–474, https://doi.org/10.1002/med.21418.

[46] H.A. Abou-Zied, B.G.M. Youssif, M.F.A. Mohamed,