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Isoxazole Derivatives: The Search for Anticancer Drugs

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Abstract Over the last seven years, there has been a continuous interest towards structural modification of isoxazole heterocycle in order to develop anticancer agents. This quest culminated in the syntheses of a large number of compounds and their evaluation against several cancer cell lines. In this paper, emphasis is made on reporting the synthesis and anticancer studies of selected compounds that exhibited a spectacular activity against the different cancer cell lines.

Keywords Isoxazoles, hybrid molecules, synthesis, anticancer activity

Introduction

Cancer represents the second leading cause of death after cardiovascular diseases worldwide [1, 2]. Regrettably however, the search for novel structures that can act as a more potent, selective, and reliable anticancer agents continued to pose a major challenge to medicinal chemists [3]. This is because of the major limitations, such as lack of selectivity of the existing drugs for cancer cells, which bring about unwanted side effects, and acquisition of multiple-drug resistance by the cancer cells [4]. Consequently, the need to discover and develop new lead compounds to combat this menace is of paramount importance. Isoxazole moiety is a promising heterocycle that feature in various synthetic compounds which demonstrate a number of biological activities. Several works revealed that isoxazole containing compounds exhibits antioxidant, antibacterial [5], anti-ageing [6], antiviral [7], analgesic, anti-inflammatory [8], anti-fungicidal [9] and anti-tubercular activities [10]. In this mini-review, an attempt is also made to showcase some representative isoxazole compounds with potential anticancer activity reported within the years 2010-2017.

Synthesis

Inspired by the structure of Gefitinib (Figure 1) which is a clinical drug for the treatment of cancer, Yong et al., [11] synthesised fourteen novel isoxazole-moiety-containing quinazoline derivatives with the potential of having a better activity and selectivity towards the cancer cells.

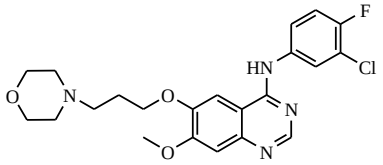
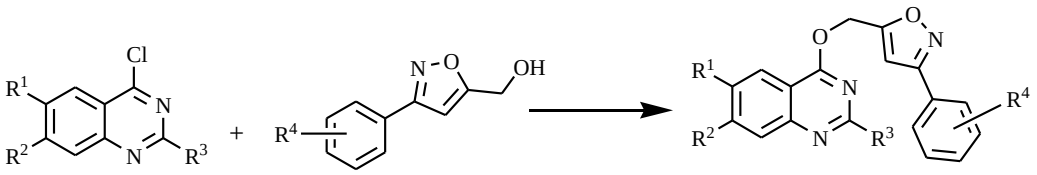


Figure 1: Gefitinib

The starting quinazoline compounds **1a-n** were commercially obtained and thus directly reacted with the corresponding isoxazoles **2a-n** which were prepared according to the reported procedure [12]. The coupling of the isoxazoles **2a-n** was achieved in the presence of triethylamine at a cold to room temperature for 8 hours to yield the hybrid products **3a-n** in excellent to good yields (Scheme 1).

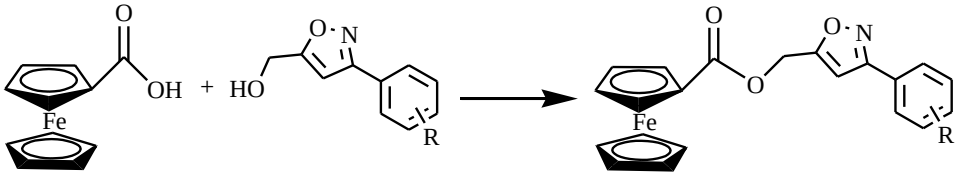


1a-n **2a-n** **3a-n**

a: $R^1 = R^2 = H$, $R^3 = Ph$, $R^4 = H$
b: $R^1 = R^2 = H$, $R^3 = Ph$, $R^4 = 4\text{-}CH_3$
c: $R^1 = R^2 = H$, $R^3 = Ph$, $R^4 = 4\text{-}OCH_3$
d: $R^1 = R^2 = H$, $R^3 = Ph$, $R^4 = 2\text{-}Cl$
e: $R^1 = R^2 = H$, $R^3 = Ph$, $R^4 = 4\text{-}Cl$
f: $R^1 = R^2 = H$, $R^3 = Ph$, $R^4 = 2,4\text{-}diCl$
g: $R^1 = R^2 = H$, $R^3 = Ph$, $R^4 = 4\text{-}Br$
h: $R^1 = R^2 = OCH_3$, $R^3 = Cl$, $R^4 = H$
i: $R^1 = R^2 = OCH_3$, $R^3 = Cl$, $R^4 = 4\text{-}CH_3$
j: $R^1 = R^2 = OCH_3$, $R^3 = Cl$, $R^4 = 4\text{-}OCH_3$
k: $R^1 = R^2 = OCH_3$, $R^3 = Cl$, $R^4 = 2\text{-}Cl$
l: $R^1 = R^2 = OCH_3$, $R^3 = Cl$, $R^4 = 4\text{-}Cl$
m: $R^1 = R^2 = OCH_3$, $R^3 = Cl$, $R^4 = 2,4\text{-}diCl$
n: $R^1 = R^2 = OCH_3$, $R^3 = Cl$, $R^4 = 4\text{-}Br$

Scheme 1.

The aromaticity, chemical stability, and low toxicity of ferrocene attracted the attention of Yong et al., [13] to utilize this pharmacopore in the synthesis of seven new isoxazole moiety containing ferrocene derivatives. Ferrocene carboxylic acid **4** in the presence of Dicyclohexylcarbodiimide (DCC) and Dimethylaminopyridine (DMAP) was reacted with various isoxazole intermediates **5a-g** which were obtained using a reported procedure [12] to furnish the desired hybrid molecules **6a-g** at a mild temperature within 40 min (Scheme 2).

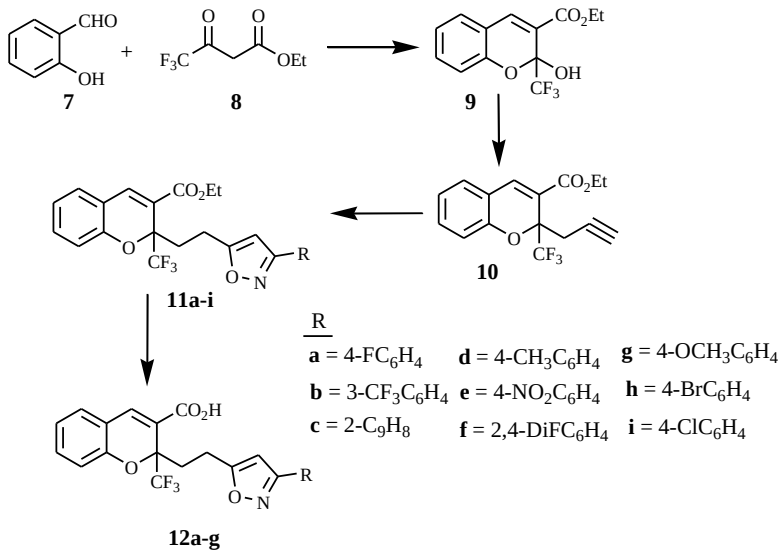


4 **5a-g** **6a-g**

a = H **b** = $4\text{-}CH_3$ **c** = $4\text{-}OCH_3$ **d** = 2-Cl **e** = 4-Cl **f** = 2,4-diCl **g** = 4-Br

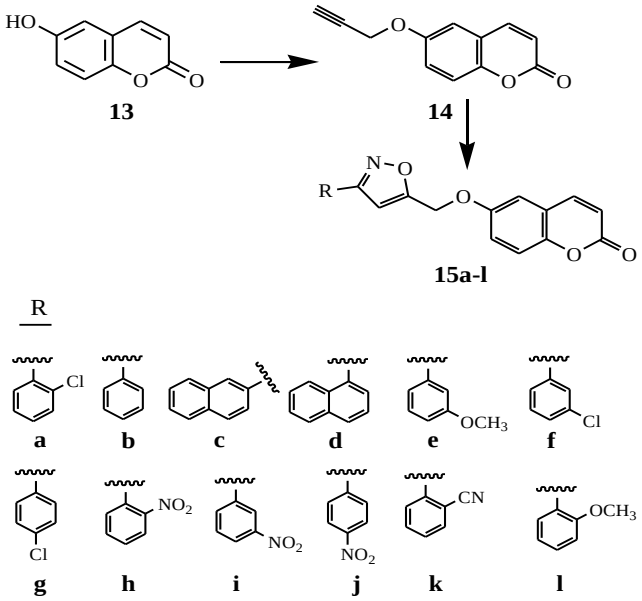
Scheme 2.

In an effort to synthesise a series of novel isoxazole functionalized *2H*-Chromene, Reddy et al., [14] reacted salicyldehyde **7** with ethyl-4,4,4-trifluoroacetoacetate **8** using piperidine as a base and obtained 2-hydroxy-2-trifluoromethyl chromene-3-carboxylate ethyl ester **9**. Compound **9** was then reacted with propagyl bromide in acetone using K_2CO_3 as a base and obtained *O*-propargylated chromene derivative **10** which was further reacted with various aryl aldoximes to furnish isoxazole functionalized *2H*-Chromene intermediates **11a-g**. Acid hydrolysis of **11a-g** yielded the final products **12a-g** (Scheme 3).



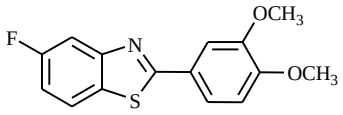
Scheme 3.

A new series of diverse isoxazoles linked 6-hydroxycoumarin were reported by Rehman et al., [15]. The starting 6-hydroxycoumarin **13** was isolated from the root parts of *Prangos pabularia* which was purified by repeated column chromatography to achieve 98% purity. Compound **13** was subjected to alkylation at the hydroxyl position using propargyl bromide in the presence of a base (K_2CO_3) to yield 6-(prop-2-ynyloxy)-2H-chromen-2-one **14**. The desired isoxazoles were obtained by reacting **14** with various aryl aldoximes to obtain **15a-l** (Scheme 4).



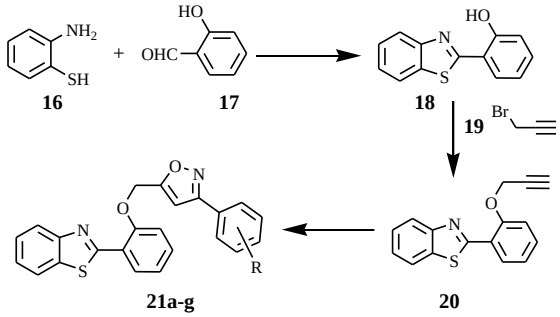
Scheme 4.

Modified benzothiazole nucleus such as PMX 610 (Figure 2) was reported to exhibit potent and selective in vitro antitumor properties [16]. This prompted Kumbhare et al., [17] to synthesise a number of isoxazoles linked 2-phenyl benzothiazole. Thus thiophenol **16** and 2-hydroxybenzaldehyde **17** prepared by the literature method [18] were reacted to furnish the 2-(2´-hydroxyphenyl) benzothiazole **18**. Alkylation of **18** with propagyl bromide **19** yielded compound **20** which was further reacted with the various oximes to obtain the final compounds **21a-g** (Scheme 5).



PMX610

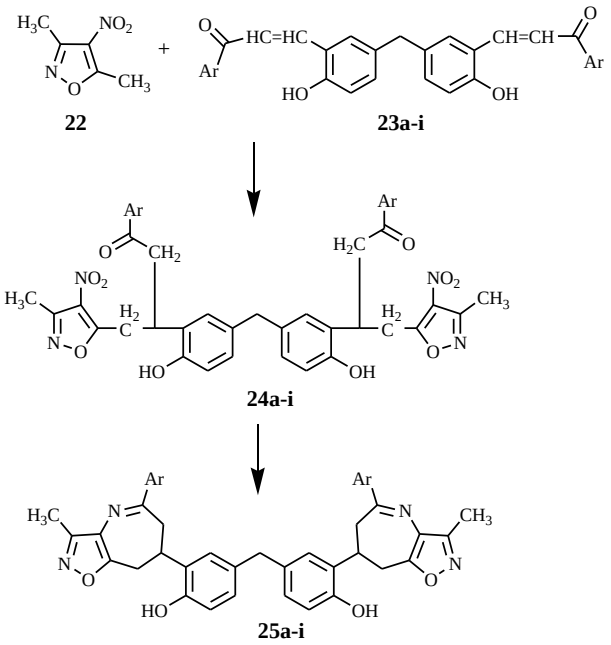
Figure 2.



R

a = 4-CH_3 **b** = 4-OCH_3 **c** = 2-F **d** = 4-Cl **e** = 4-Br **f** = 4-OCF_3 **g** = 3,4,5-OCH_3

Scheme 5.



Ar

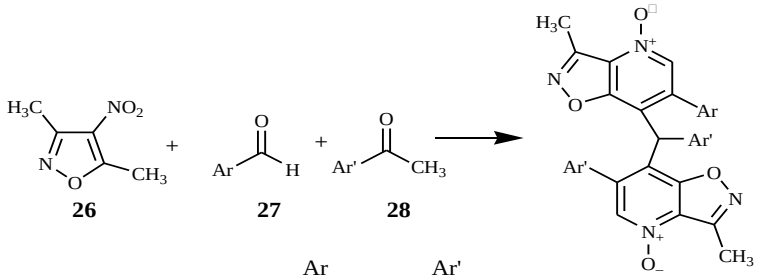
a = C_6H_5 ; **b** = 4-BrC_6H_4 ; **c** = 4-$NO_2C_6H_4$; **d** = 4-ClC_6H_4 ;
e = 4-$OCH_3C_6H_4$; **f** = 4-$CH_3C_6H_4$; **g** = 2-OHC_6H_4 ;
h = 2-furyl ; **i** = 2-thienyl

Scheme 6.

Rajanarendar et al., [19] conceived the idea of introducing two isoxazoleazepine moieties in a single molecular skeleton in order to develop compounds with better pharmacological potential. The synthesis was accomplished as shown in Scheme 6. It started with the Michael addition of 3,5-dimethyl-4-nitroisoxazole **22** with the various methylene bis-chalcones **23a-i** in the presence of piperidine to furnish the corresponding methylene bis-[3-methyl-4-

nitro-5-(1-phenyl-3-(2-hydroxyphenyl)-1-keto-4-n-butyl)isoxazoles **24a-i**. Reductive cyclisation of the Michael adducts on treatment with $SnCl_2$-MeOH yielded compounds **25a-i**.

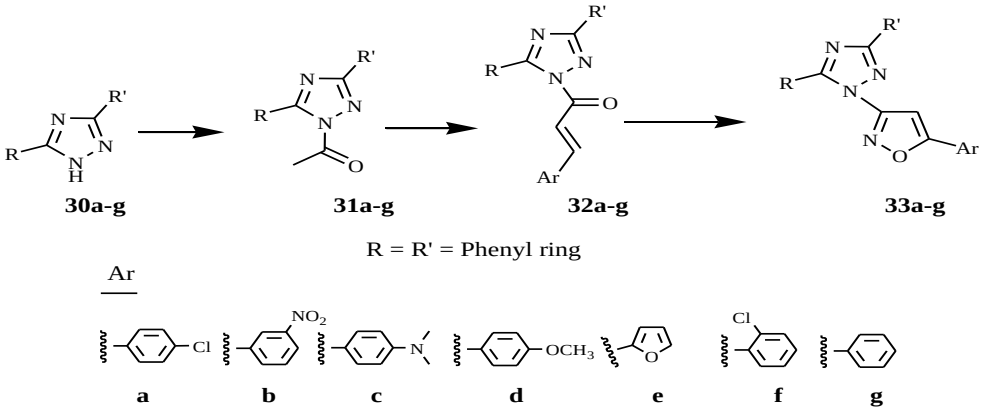
Owing to the potential of isoxazole-*N*-oxides as anticancer agents [20], Rajanarendar et al., [21] undertook a multicomponent synthesis of novel arylmethylene bis-isoxazolo[4,5-*b*] pyridine-*N*-oxides with the hope of developing molecules which will offer a better anticancer activity. Thus 3,5-dimethyl-4-nitroisoxazole **26**, aromatic aldehydes **27**, and aromatic ketones **28** were reacted in the presence of piperidine to afford the respective compounds **29a-j** (Scheme 7).



	Ar	Ar'
a	C_6H_5	C_6H_5
b	2-$CH_3C_6H_4$	C_6H_5
c	2-$CH_3OC_6H_4$	C_6H_5
d	2-ClC_6H_4	C_6H_5
e	4-ClC_6H_4	C_6H_5
f	C_6H_5	4-$CH_3C_6H_4$
g	C_6H_5	4-$CH_3OC_6H_4$
h	C_6H_5	2-HOC_6H_4
i	C_6H_5	4-ClC_6H_4
j	C_6H_5	4-$NO_2C_6H_4$

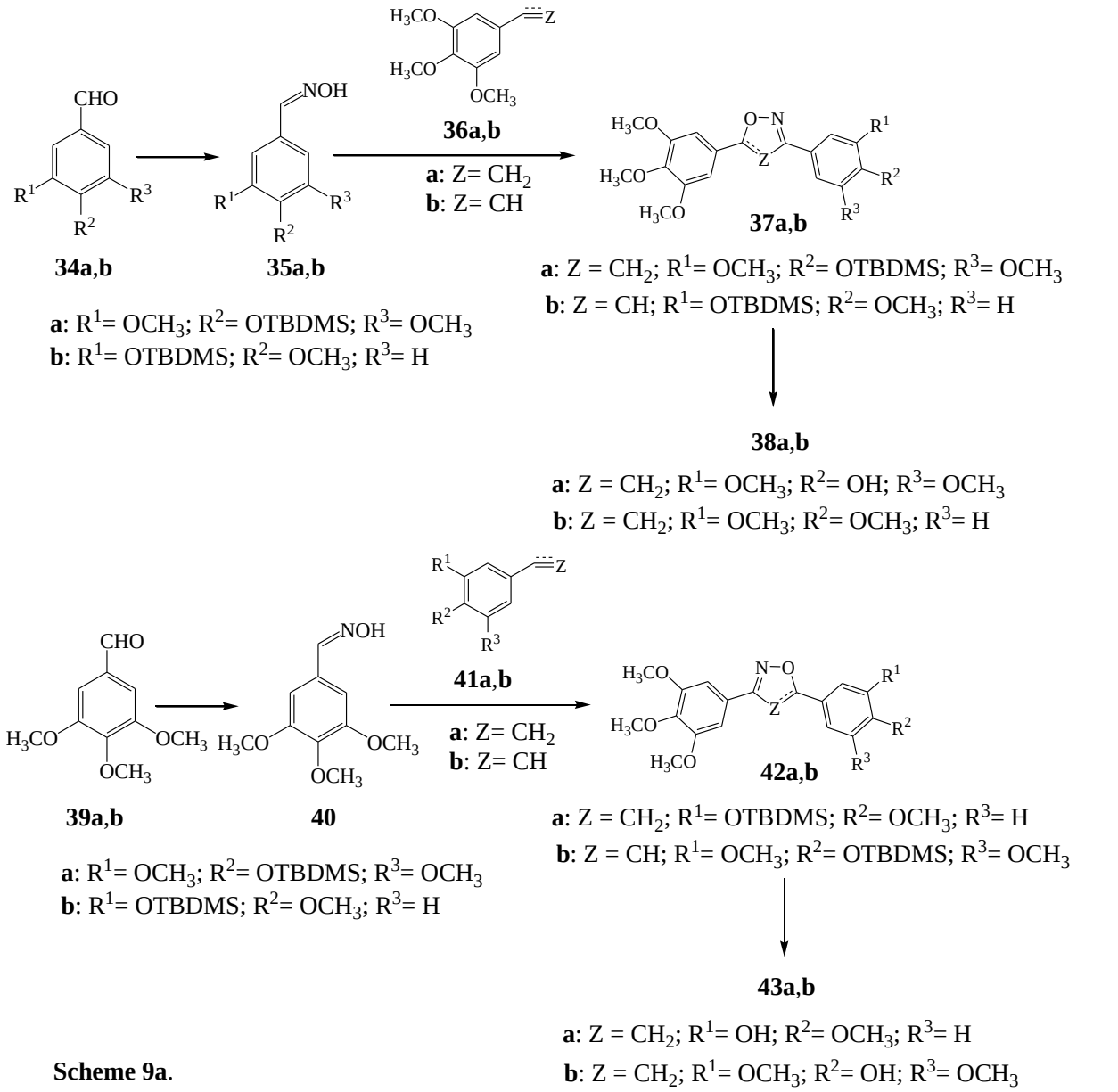
Scheme 7.

A new class of isoxazole derivatives containing 1,2,3-triazole moiety were reported by Khanage et al., [22] as described in Scheme 8. Reacting the appropriate 1,2,3-triazoles **30a-g** with acetic anhydride and conc. H_2SO_4 furnished the acetylated compounds **31a-g**. These were easily converted to the corresponding chalcones **32a-g** by reacting them with the substituted aryl adehydes. Cyclisation of the chalcones with hydroxylamine hydrochloride in basic medium afforded the required isoxazole derivatives **33a-g**.



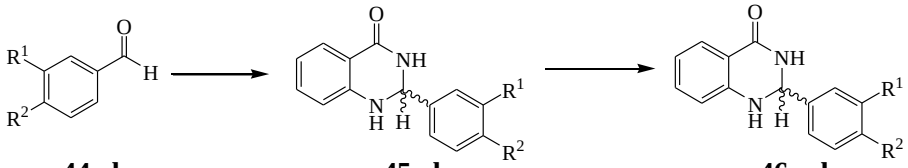
Scheme 8.

Kamal et al., [23] adopted a convergent synthesis approach to obtain a series of new 3,5-diaryl isoxazoline/isoxazole linked 2,3-dihydro quinazolinone hybrids that could offer a synergistic anticancer activity. The first segment of the synthesis (Scheme 9a) began with the preparation of oximes **35a,b** and **40**.Thus aldehydes **34a,b** and **39a,b** were reacted with hydroxylamine hydrochloride to afford the corresponding oximes **35a,b** and **40** in high yield. Reaction of aldehydes **39** and **34b** respectively with methyltriphenyl phosphonium bromide, in the presence of sodium hydride furnished the respective olefins **36a** and **41a**. These olefins were then coupled respectively with **35a** and **40** to afford compounds **37a** and **42a**. Equally, isoxazoles **37b** and **42b** were prepared by employing alkynes **36b** and **41b** and oximes (**35b** and **40**). Deprotection of these compounds (**37a,b** and **42a,b**) with tetrabutylammonium bromide yielded the desired precursors **38a,b** and **43a,b**.



Scheme 9a.

The second segment of the synthesis (Scheme 9b) dealt with the preparation of the 2,3-dihydroquinazolinone. Treatment of compounds **44a,b** with benzamide in the presence of *N,N*-dimethylacetamide (DMAC) gave compounds **45a,b** respectively. Subsequent etherification of these compounds with dibromoalkanes using K_2CO_3 in DMF furnished the 2,3-dihydroquinazolinone precursors **46a-d**.



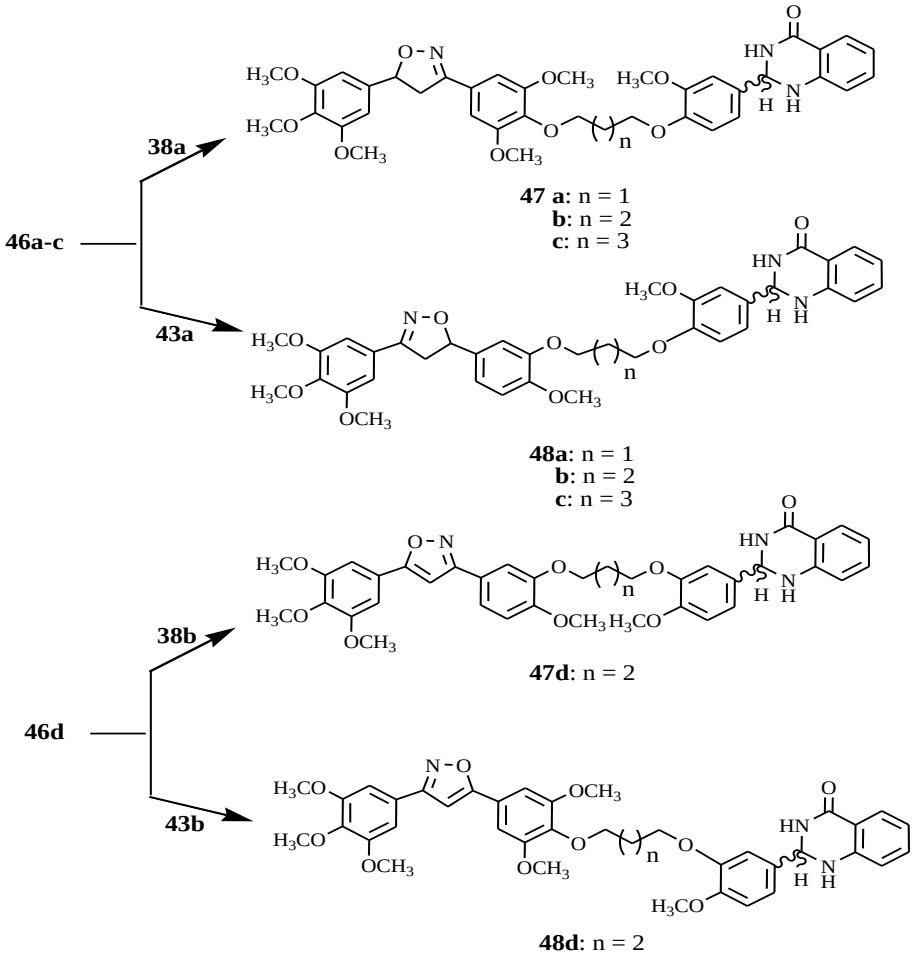
44a,**b** **45a**,**b** **46a-d**

44a: R^1= OCH_3; R^2= OH
44b: R^1= OH; R^2= OCH_3

46a:R^1= OCH_3; R^2= -O-$(CH_2)_3$Br
b:R^1= OCH_3; R^2= -O-$(CH_2)_4$Br
c:R^1= OCH_3; R^2= -O-$(CH_2)_5$Br
d:R^1= -O-$(CH_2)_4$Br; R^2= OCH_3

Scheme 9b.

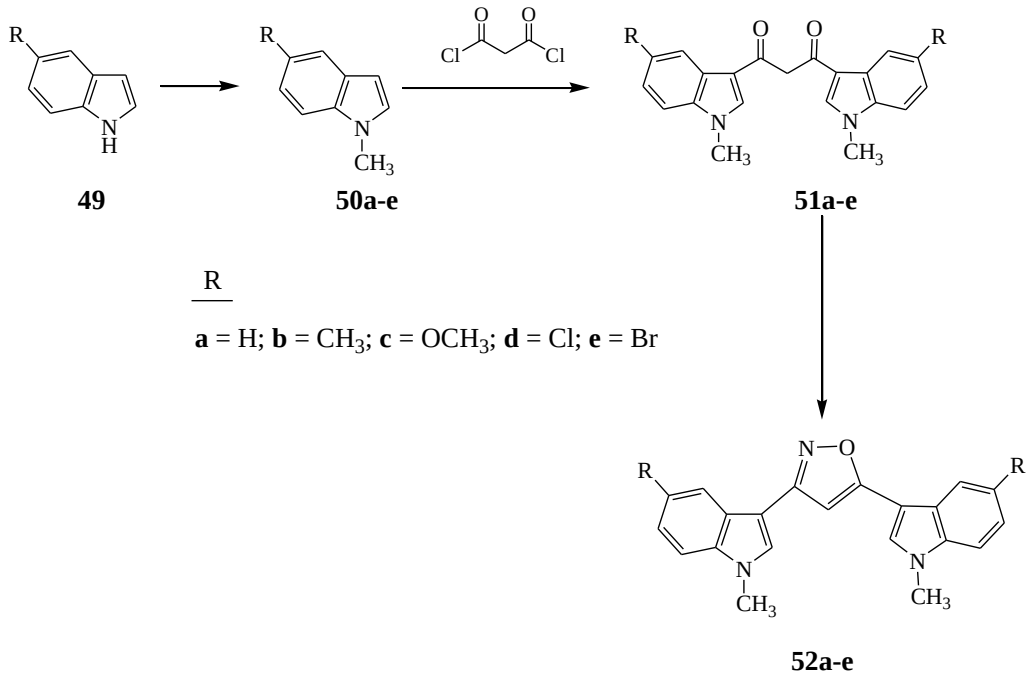
Finally the synthesis of the hybrid molecules was achieved from the compounds **46a-d** and 3,5-diarylisoxazoline/isoxazole precursors **38a,b** and **43a,b** using K_2CO_3 in DMF (Scheme 9c).



Scheme 9c.

In continuation of their effort to develop new bis-indolyl-5-membered heterocycles with potential anticancer activity, Diana et al., [24] synthesised a series of 3,5-bis(3-indolyl)-isoxazoles as shown in Scheme 10. It started with the methylation of indole derivatives **49a-e** using potassium *t*-butoxide, tris[2-(2-methoxyethoxy)ethyl]amine as catalyst, and methyl iodide to obtain the *N*-methyl derivatives **50a-e**. Addition of malonyl-dichloride in a Friedel-

Crafts fashion yielded the symmetrical 1,3-diketones **51a-e**. These were reacted with hydroxylamine hydrochloride in refluxing TEA/THF to furnish the corresponding 3,5-bis(3-indolyl)-isoxazoles **52a-e**.



Scheme 10.

Anticancer Activity

Compounds **3a-n** were screened *in vitro* against lung cancer A549, colorectal cancer HCT116 and breast cancer MCF-7 cell lines using gefitinib as reference drug by MTT method [25]. Comparison of the IC_{50} values of the compounds revealed that **3d**, **3i**, **3k**, and **3m** have the most potent and wide spectrum activity against the three cancer cell lines. The IC_{50} values ranges from 0.11 – 77.05µM suggesting that any of these can be regarded as a potential candidate for the development of anticancer drugs. Preliminary anticancer evaluation of compounds **6a-g** was also performed using the MTT assay [25] against the aforementioned cancer lines using gefitinib as the reference drug. The result strikingly shows that **6d** (IC_{50} = 7.47×10^{-4}) is 23 962-fold more active than gefitinib against the A549 cell line, and 5 904-fold (IC_{50} = 3.65×10^{-3}) more active than gefitinib against the HCT116 cell line. This encouraged the workers to continue with further study of the anticancer efficacy of this promising compound. Using the MTT method [25], compounds **11a-i** and **12a-g** were screened *in vitro* against four human cell lines, viz. A549-Lung cancer (CCL-185), MCF7-Breast cancer (HTB-22), DU145-Prostate cancer (HTB-81), HeLa-Cervical cancer (CCL-2). Comparison of the IC_{50} values of the compounds with the positive control (5-Fluorouracil) revealed that **11a**, **11b**, **11i**, **12a** and **12f** showed activity at <50µM against all the cell lines. Cytotoxicity screening of 6-Hydroxycoumarin **13**, its propargylated product **14** and the isoxazole derivatives **15a-l** was carried out using MTT method [25] against a panel of five different human cancer cell lines, namely prostate (PC-3), colon (HCT-116, Colo-205), leukemia (HL-60) and lung (A-549). BEZ-235 was used as positive control. Among the compounds, **15h** and **15k** showed the best activity with the IC_{50} of 8.2 and 13.6µM against prostate (PC-3) cancer cell line respectivley. Evaluation of cytotoxic activity of compounds **21a-g** against human adenocarcinoma (A549), colon (Colo-205), and breast carcinoma (MCF-7) cell lines was carried out using MTT assay [25]. Interestingly all these compounds showed a potent broad spectrum activity against all the three cancer cell lines. Particularly compound **21a** which shows almost similar activity (IC_{50} = 14.16µM) as the positive control, PMX 610 (13.94µM) against Colo-205 cancer cell line. Compounds **25a-i** were evaluated for their *in vitro* anticancer activity against two breast cancer cell lines, MCF-7 (ER-positive), MDA-MBA-231 (ER-positive), and human kidney cancer epithelial cell line HEK-293 according to MTT assay using Cisplatin (DPP) as reference drug [25]. In comparison to the reference

drug, compounds **25h** and **25i** showed a better activity towards all the three cancer cell lines. The rest of the compounds also exhibited some moderate anticancer activity against the three cell lines. Following the MTT assay [25], compounds **29a-j** were screened *in vitro* for anticancer activity against human cell cultures HeLa (cervical), Ehrlich Ascites Carcinoma (EAC), and MCF-7 (breast). Cispalatin (DPP) was used as the reference drug. Among all the compounds tested, **29a** and **29h** appeared to be the most cytotoxic in all the three cell lines. This led to further studies on their in vivo antitumor properties in EAC-bearing mice using liquid tumor model [21]. The result also suggests that **29a** is more active in *in vivo* cytotoxic studies and hence calls for simple modification of the structure to develop a potent anticancer drug. Preliminary anticancer screening of compound **33a**, **33c**, **33d**, **33e** and **33f** was performed according to the US NCI protocol. The assay was carried out at a single concentration of 10^{-5}M towards the panel of approximately sixty cancer cell lines derived from nine different cancer types: leukemia, lung, colon, CNS, melanoma, ovarian, renal, prostate, and breast cancers. Among the compounds tested, **33d** and **33e** appeared to have the most prominent effect and significant anticancer activity. Using the same US NCI protocol, **47c** was evaluated for a pre-test against a panel of the aforementioned sixty cancer cell lines derived from nine different cancer types. The astonishing activity of **47c** led to its selection for further assay over a 5-log dose range (10^{-4} - 10^{-8}M). Interestingly, compound **47c** demonstrated significant activity against RPMI-8226 (leukemia) and SK-MEL-5 (melanoma) cell lines. This encouraged the evaluation of **47c** along with **47a**, **47b**, **47d** and **48a-d** using the Sulforhodamine B (SRB) method against selected human cancer cell lines. The positive control, Adriamycin [23] exhibited activity in the range 0.13 – 0.19µM while **48a-d** showed promising anticancer activity against A549 cell line with the GI_{50} values in the range 0.15 – 0.18µM. The most potent **47c** was again subjected to the MTT assay [25] along with its left side conjugate partner **38a** as well as the right side conjugate partner **46c**. Apparently, **47c** showed enhanced cytotoxicity at 5µM against MCF-7 cell lines in comparison to the conjugate partners. In order to understand the mechanism of action of **47c**, biological assays relating to the cell cycle aspects and tubulin depolymerisation activity were examined. The data suggest that **47c** has the ability to cause G2/M cell cycle arrest. Also **47c** showed the disruption of microtubules as well as fragmentation of nuclei. Moreover, compound **47c** was identified as an effective cyclin B1 and CDK153 inhibitor. Using a monolayer cell survival and proliferation assay [26] compounds **52a-e** were screened for *in vitro* antitumor activity in a panel of 10 humor cell lines. Adriamycin was used as the positive control. The most active compound **52a** was further evaluated in a panel of 29 cell lines, exhibiting mean IC_{50} value of 53.2µM. In particular, compound **52a** showed a high level of selectivity toward A549 and LXFA 629L (lung), as well as UXF 1138L (uterus body).

Conclusion

The isoxazole derivatives captured in this mini-review are carefully selected to reflect those compounds reported within the last seven years which demonstrated a promising activity against various cancer cell lines. Mostly the compounds were constructed as hybrid molecules in order to confer a synergistic effect on the tested cancer cell lines. Generally, the *in vitro* activity of these compounds is so brilliant that further structural modification could lead to the discovery of anticancer agents.

Acknowledgement

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References

1. Gibbs, J. B. (2000). Mechanism-based target identification and drug discovery in cancer research. *Science*, 287(5460):1969-1973.
2. Unger, C. (1997). New therapeutic approaches in cancer treatment. *Drugs of the Future*, *22*(12):1337-1345.
3. Heffeter, P., Jakupec, M. A., Körner, W., Wild, S., von Keyserlingk, N. G., Elbling, L., Zorbas, H., Korynevska, A., Knasmuller, S., Sutterluty, H., Micksche, M., Keppler, B. K., & Berger, W. (2006). Anticancer activity of the lanthanum compound [tris (1, 10-phenanthroline) lanthanum (III)] trithiocyanate (KP772; FFC24). *Biochemical Pharmacology*, 71(4):426-440.
4. Menta, E., & Palumbo, M. (1997). Novel antineoplastic agents. *Expert Opinion on Therapeutic Patents*, 7(12):1401-1426.
5. Musad, E. A., Riyaz, M., Bahjat, A. S., Bannikuppe, S. V., & Rai, K. M. L. (2011). Synthesis and evaluation of antioxidant and antibacterial activities of new substituted bis (1, 3, 4-oxadiazoles), 3, 5-bis (substituted) pyrazoles and isoxazoles. *Bioorganic & Medicinal Chemistry Letters*, 21(12):3536-3540.
6. Koufaki, M., Theano, F., Marianna, K., Georgios, A. H., Efstathios, S. G., & Niki, C. (2014). Microwave-assisted synthesis of 3, 5-disubstituted isoxazoles and evaluation of their anti-ageing activity. *European Journal of Medicinal Chemistry*, 83:508-515.
7. Li, F., Yanmei, H., Yuanxiang, W., Chunlong, M., & Jun, W. (2017). Expeditious Lead Optimization of Isoxazole-Containing Influenza A Virus M2-S31N Inhibitors Using the Suzuki–Miyaura Cross-Coupling Reaction. *Journal of Medicinal Chemistry*, 60(4):1580-1590.
8. Kankala, S., Ranjith, K. K., Prasad, G., Niranjan, Th., Srinivas, N., Mohan, R. G., Hanmanthu, G., Mukkanti, K., Ravinder, V., & Chandra, S. V. (2013). Regioselective synthesis of isoxazole–mercaptobenzimidazole hybrids and their in vivo analgesic and anti-inflammatory activity studies. *Bioorganic & Medicinal Chemistry Letters*, 23(5): 1306-1309.
9. Brahmayya, M., Battula, V., Dasari, K., Sankuratri, D., Uppuleti, V. P., Thoti, D., & Ramtej, M. (2013). Synthesis and fungicidal activity of novel 5-aryl-4-methyl-3yl (imidazolidin-1yl methyl, 2-ylidene nitro imine) isoxazoles. *Journal of Pharmacy Research*, 7(6):516-519.
10. Vergelli, C., Cilibrizzi, A., Crocetti, L., Graziano, A., Dal Piaz, V., Wan, B., Wang, Y., Franzblau, S. & Giovannoni, M. P. (2013). Synthesis and Evaluation as Antitubercular Agents of 5-Arylethenyl and 5-(Hetero) aryl-3-Isoxazolecarboxylate. *Drug Development Research*, 74(3):162-172.
11. Yong, J. –P., Lu, C. –Z. & Wu, X. (2015). Potential Anticancer Agents. I. Synthesis of Isoxazole Moiety Containing Quinazoline Derivatives and Preliminarily *in vitro* Anticancer Activity. *Anti-Cancer Agents in Medicinal Chemistry*, 15(1):131-136.
12. Liu, L. J., Yong, J. P., Dai, X. J., Jia, J., Wang, X. Z., Wang, J. W. (2006). Synthesis of novel isoxazole contained glcyrrhetinic acid derivatives. *Chemical Journal of Chinese Universities*, 27:1669-1672.
13. Yong, J. –P., Lu, -C & Wu, X. (2014). Synthesis of isoxazole moiety containing ferrocene derivatives and preliminarily in vitro anticancer activity. *Medicinal Chemistry Communication*, 5(7):968–972.
14. Reddy, K. R., Rao, P. S., Dev, G. J., Poornachandra, Y., Kumar, C. G., Rao, P. S., Narsaiah, B. (2014). Synthesis of novel 1,2,3-triazole/isoxazole functionalized 2H-Chromene derivatives and their cytotoxic activity. *Bioorganic & Medicinal Chemistry Letters*, 24(7):1661–1663
15. Rehman, S. -U, Rahman, M. –U., Tripathi, V. K., Singh, J., Koul, T. A. S., Farooq, S., & Kaul, A. (2014). Synthesis and biological evaluation of novel isoxazoles and triazoles linked 6-hydroxycoumarin as potent cytotoxic agents. *Bioorganic & Medicinal Chemistry Letters*, 24(17):4243–4246.
16. Aiello, S., Geoffrey, W., Erica, L. S., Hachemi, K., Rana, B., David, R. B., Malcolm, F. G. S., Charles, S. M., Tracey, D. B., & Andrew, D. W. (2008). Synthesis and biological properties of benzothiazole, benzoxazole, and chromen-4-one analogues of the potent antitumor agent 2-(3, 4-dimethoxyphenyl)-5-fluorobenzothiazole (PMX 610, NSC 721648)(1). *Journal of Medicinal Chemistry*, 51(16):5135-5139.
17. Kumbhare, R. M., Kosurkar, U. B., Ramaiah, M. J., Dadmal, T. L., Pushpavalli, S. N. C. V. L., & Bhadra. M. P-. (2012). Synthesis and biological evaluation of novel triazoles and isoxazoles linked 2-phenyl

benzothiazole as potential anticancer agents. *Bioorganic & Medicinal Chemistry Letters*, 22(17):5424–5427.

18. Bahrami, K., Mehdi, K. M., & Fardin, N. (2008). Mild and highly efficient method for the synthesis of 2-arylbenzimidazoles and 2-arylbenzothiazoles. *The Journal of Organic Chemistry*, 73(17):6835-6837.
19. Rajanarendar, E., Reddy, M. N., Krishna, S. R., Reddy, K. G., Reddy, Y. N., & Rajam M. V. (2012). Design, synthesis, in vitro antimicrobial and anticancer activity of novel methylenebis-isoxazolo[4,5-*b*]azepines derivatives. *European Journal of Medicinal Chemistry*, 50:344-349.
20. Maksimovic-Ivanic, D., Mijatovic, S., Harhaji, L., Miljkovic, D., Dabideen, D., Cheng, K. F., Mangano, K., Malaponte, G., Al-Abed, Y., Libra, M., Garotta, G., Nicoletti, F., & Stosic-Grujicic, S. (2008). Anticancer properties of the novel nitric oxide-donating compound (S, R)-3-phenyl-4, 5-dihydro-5-isoxazole acetic acid-nitric oxide in vitro and in vivo. *Molecular Cancer Therapeutics*, 7(3):510-520.
21. Rajanarendar, E., Rajua, S., Reddy, M. N., Krishna, S. R., Kiran, L. H., Reddy, A. R. N., Reddy, Y. N. (2012). Multi-component synthesis and in vitro and in vivo anticancer activity of novel arylmethylene bis-isoxazolo[4,5-*b*]pyridine-N-oxides. *European Journal of Medicinal Chemistry*, 50:274-279.
22. Khanage, S., Mohite, P., Pandhare, R., & Raju, A. (2012). Synthesis and pharmacological evaluation of isoxazole derivatives containing 1,2,4-triazole Moiety. *Marmara Pharmaceutical Journal*, 16(2):134-140.
23. Kamal, A., Bharathi, E. V., Reddy, J. S., Ramaiah, M. J., Dastagiri, D., Reddy, M. K., Viswanath, A., Reddy, T. L., Shaik, T. B., Pushpavalli, S. N. C. V. L., & Bhadra, M. P. (2011). Synthesis and biological evaluation of 3,5-diaryl isoxazoline/isoxazole linked 2, 3-dihydroquinazolinone hybrids as anticancer agents. *European Journal of Medicinal Chemistry*, 46(2):691-703.
24. Diana, P., Carbone, A., Barraja, P., Kelter, G., Fiebig, H. –H., & Cirrincione, G. (2010). Synthesis and antitumor activity of 2,5-bis(3´-indolyl)-furans and 3,5-bis(3´-indolyl)-isoxazoles, nortopsentin analogues. *Bioorganic & Medicinal Chemistry*, 18(12):4524–4529.
25. Botta, M., Armaroli, S., Castagnolo, D., Fontana, G., Perad, P., & Bombardelli, E. (2007). Synthesis and biological evaluation of new taxoids derived from 2-deacetoxytaxinine J. *Bioorganic & Medicinal Chemistry Letters*, 17(6):1579-1583.
26. Dengler, W. A., Schulte, J., Berger, D. P., Mertelsemann, R., & Fiebig, H. H. (1995). Development of a propidium iodide fluorescence assay for proliferation and cytotoxicity assays. *Anticancer Drugs*, 6(4):522-532.